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Review Article

Aflatoxins in Mozambique: etiology, epidemiology and control

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Abstract: Mozambique is endemic to aflatoxigenic *Aspergillus* but the country has to heavily rely on foreign research to deduct what is happening locally. There is some information produced by local scholars and institutions but it needs to be "tied" together. This review briefly synthetizes the country's major findings in relation to the toxin's etiology, epidemiology, detection and control, discussing and meta-analyzing them as far as they allow. The causes and foods affected are the same as in most tropical countries, the toxin is widespread and the level of exposure is high. Regarding the control, it is still marginal but some institutions have driven efforts in this direction. Learning from other countries is still the best approach to take, as the solutions are probably the same for most places.

Keywords: aflatoxins; Mozambique; etiology; epidemiology; control

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1. Introduction

The information on AFB_1 in Mozambique is clustered in sporadic, scarcely related reports, and there is very low awareness outside academic circles. Warth, *et al.* [1] mentioned the urge for comprehensive data in mycotoxins. There is a review by Ferrão, *et al.* [2] about mycotoxins in Southern Africa. It is introductory and generic in content, probably because it explores a very broad topic. There is little research about AFB_1 because its surveillance and control are generally costly. Indeed, Harmsen, *et al.* [3] presented an interview with dr. David Mariote, Eduardo Mulima and Miguel Magalhães stating a low demand for AFB_1 analysis in Nampula province, not compensating the investment in the equipment. But the author expects demand for such kind of analysis to increase in a near future.

Organized information is the key for a better understanding. The isolated clusters of scientific production need to be tied in a concise document for the current and future generations. Well-mapped information on aflatoxins in Mozambique will show where are the gaps to be filled and the next steps for the academia, industry and civil society. It also brings contradictions and unclear parts of the research to the surface. For example, most authors make people perceive groundnuts as the biggest and sometimes sole source of aflatoxins [4,5], while Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] found more in maize than in groundnuts. There might be some bias just because most AFB_1 research is on groundnuts because of trade requests.

This paper aims to present the causes, distribution and control of aflatoxin exposure in Mozambique according to the studies performed in the country so far.

2. Etiology and contamination

2.1. Etiology

In Mozambique, aflatoxins are produced by the same molds found in other places: *A. flavus* and *A. parasiticus* [1,6]. Augusto, Atehnkeng, Akello, Cotty and Bandyopadhyay [6] mentioned the same fungi, stressing *A. flavus* L-strain and *A. parasiticus* as the most abundant in the center and north of the country, but they also found *A. flavus* S-strain and *A. tamari*. Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] found AFB_1 in their samples, and it suggested the presence of *A. parasiticus* or *A. flavus* strain SBG.

Most studies from Mozambique are focused on AFB_1 or do not distinguish the aflatoxins. Few, such as the survey one mentioned by Baquete and Freire [7] and van Wyk, Van der Merwe, Subrahmanyam and Boughton [4], also detected AFB_2. AFG_1 and AFG_2 in Mozambican food. The lack of this depth in most researches is probably due to the overall priority of simply analyzing the safety. Despites the differences in toxicity, they all are harmful and should be avoided.

2.2. Commodities

Aflatoxins can be found in several commodities. The hosts can range from cotton, wheat and sorghum [8,9] to rice [10] and others. Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] added millet, feed and feed waste to the list. Virtually all sorts of cereals, grains and their derivatives are susceptible to contamination. There is also beer made from fresh cassava, but it was never analyzed in Mozambique [2]. However, most studies in Mozambique are focused on maize and groundnut. Cassava has also received some attention, though very few times [11-13]. These crops are better known for their high commercial value and a history of confirmed cases. Yet, there is still a need to better clarify the data on the toxin's prevalence in maize and groundnut in Mozambique Bandyopadhyay and Dubois [14].

However, the intense focus on only two or three major crops ends up neglecting many more, In his book of Mozambican water and foods, Casadei [13] reports aflatoxin contamination in rice. Sorghum, maize, sweet corn, corn flour, wheat, beans, groundnuts, sesame, dry cassava, cassava flour, forage, beer and other foods. Baquete and Freire [7] said there were more commodities, being 17 in total. All but sorghum, sesame, dry cassava and beer were contaminated by over 5 g/kg. Among them, maize, corn products, rice, groundnuts and beer had over 50% of samples contaminated, and from these, groundnuts had the highest median aflatoxin content (49 g/kg). These observations stress groundnuts as the prime source of aflatoxins and maize among the major. Yet, the remaining commodities should not be underestimated.

The publications above might be outdated because the country's economic situation is not the same now [15]. Also, many more people have unprecedented access to higher levels of education and information nowadays. It would not be unrealistic to assume nothing has changed since then. Nonetheless. Augusto, Atehnkeng, Akello, Cotty and Bandyopadhyay [6] also found more recently higher levels in groundnuts in relation to maize. His study was only in the country's center and north but included Zambézia and Nampula provinces, both forming a hot spot of *Aspergillus* infestation and aflatoxin contamination. However, Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] also collected samples from Nampula but found higher prevalence (46%) and levels in maize compared to groundnuts (14%). They went even further, detecting values of aflatoxins B, G and other mycotoxins, all at higher levels in maize samples. Their differences might be related to the sampling process. Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] obtained the foods from different sources including farmers and markets, while Augusto, Atehnkeng, Akello, Cotty and Bandyopadhyay [6] worked with soil samples and foods collected from experimental fields. Maybe the foods from the market had already received some treatment such as selection of non-moldy specimens. The same applies to farmers, more focused on the production rather than research. Some other factors such as different seasons and storage can be considered.

The studies on cassava are very discrepant. Mota and Lourenço [11] found very high quantities. Casadei [13] moderated and Essers and Nout [12] nothing at all. More recent evidence from two neighboring countries, Zambia and Malawi, points towards low levels [16]. There are some distinct variables to consider: the studies were performed at different decades, covered different areas, although they all covered Nampula province, and were performed for different purposes. The only study intentionally designed to search for aflatoxins was the survey described by Casadei [13]. The

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others were more concerned about another toxins as harmful: cyanogenic compounds. This might have led the authors not to analyze more deeply the aflatoxins once they got the answer they were looking for. It is also possible that efforts to reduce the cyanogenic compounds also ended up somehow decreasing the aflatoxin content in cassava. Anyway, the aflatoxin levels in cassava and derivatives shall be regarded as inconclusive so far but it should be taken more seriously as some evidences pointed to very high levels.

There is some research on feed, mostly for poultry. Mondlane, *et al.* [17] dedicated a study to mold infestation and $AFB_{1, and}$ Walker, *et al.* [18] included feedstuff and its waste in their study on multiple toxins and commodities. The former author analyzed 69 samples from four factories, from which 65% were infested by *A. flavus* and contaminated. In some cases, the values exceeded the recommendations by the Codex Alimentarius. Walker, Pitoro, Tomo, Sitoe, Salência, Mahanzule, Donovan and Mazuze [18] found similar results, with 60% of the samples heavily contaminated. It should be expected as unfit food for human consumption is primarily fed to animals [19]. In the latter study, of the waste products was extremely contaminated, but it is understandable. The waste probably includes peels and other surface parts where the mold are more likely to grow, and maybe there is no special treatment to reduce infestation and contamination in these parts. This actually poses a problem if it is used to make manure, for example, because the molds can be rechanneled to the fields. The most important to conclude from both studies is their confirmation of high levels of aflatoxins in feed and its risks for animal health.

The study conducted by Sineque, *et al.* [20] is one example of what happens when contaminated feedstuff is given to animals. They found AFB_1 in chicken livers and gizzards from abattoirs in Maputo. The levels were low and it is apparently safe to consume these products. Nonetheless, they also demonstrated that toxins actually make into these organs and remain there for some time. Human exposure to mycotoxins is not only a function of the levels in food but also de frequency and quantity consumed. The high levels of AFB_1 are probably the cause of acute jaundice, but hepatic cancer results from a long-term effect of the aflatoxins, and it might not require high concentrations of such chemicals. It would be wise analyze the dynamics of the toxin influence on the onset of primary liver cancer.

Baquete and Freire [7] mentioned a program investigating if contaminated food is resulting in the presence of AFM_1. However, there are no further records of such event readily available. It might have been cancelled and or its results were inconclusive. Indeed, Ferrão, Bell and Fernandes [2] implied the lack of such information and recommended the studies because studies in Germany demonstrated its plausibility.

2.3. Circumstances of contamination

There is a wide international literature on how the molds make into the food and what facilitates it. However, has been difficult to directly relate the level of mold infestation with mycotoxin production [21]. Still, genetic features such as the species and strain, and environmental such as temperature and humidity, have been effectively associated toxin contamination. Most studies end up analyzing that indirectly as they describe the study area or sampling conditions, and some are designed exactly to analyze such factors. Thus, there is no shortage of information on that, though the role of each factor would be better understood if properly modeled.

Ferrão, Bell and Fernandes [2] said in Mozambique the exposure to high aflatoxin levels is due to intake of maize, cassava, peanuts and other oilseeds. This information agrees with most of the available data but it is probably victim of bias, as previously stated. Aflatoxins are available in several commodities [7,13]. Commercial and political forces are behind the abundance of knowledge about just a few crops. That is why only old surveys covered a larger variety of foods. Yet, the cash crops are indeed a good starting point to analyze the situation in Mozambique.

Natural causes can highly influence the contamination. Mozambique has a typical tropical weather and it favors the growth of *Aspergillus* in the crops [19]. Moreover, van Wyk, Van der Merwe, Subrahmanyam and Boughton [4] said that drought and temperature stress, combined with soil pests and diseases during the pre-harvest, play a major role on damaging the grain and letting

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the mold invade. There are further remarkable findings by Augusto, Atehnkeng, Akello, Cotty and Bandyopadhyay [6], based on humidity, temperature and altitude. He found high infestation in "hot, humid and low to medium altitude (50-600m)" and low in "wet, high altitude (>1000m)", not mattering which crop. It is reasonable, as one should expect less biodiversity as the altitude increases.

Though nature cannot be defied, the crop handling can highly influence the level of infestation and contamination. According to Zuza, Mondjana, Muitia and Amane [5], the combination of adverse weather and inadequate methods for harvest and post-harvest influence the contamination. The farmers, traders, processors and exporters have to be careful during lifting, drying or curing, and storing during postharvest [4]. It is important to set properly the harvesting timing, because delays expose the crops to mold infections and aflatoxin contamination [5,22]. Also, Harmsen, Bremmer and Maria [3] recommends the handlers not to store the foods in compartments too wet or in plastic bags.

The path to aflatoxin intake has four major components: *Aspergillus* infestation, aflatoxin contamination, consumers' exposure and intake. The first two were already described above. The following also deserve attention, although lacking updated and consistent information. Another thing to consider is the overall effect of the aflatoxins, not just the consumption. Ultimately, the consequences matter the most, in this case acute jaundice and hepatocellular carcinoma.

Van Rensburg, *et al.* [23] and the related studies were partially based on the analysis of foods prepared by the rural population in Inhambane province. Since high levels of toxins were found, one can imply that the handling of the food was not safe enough to prevent such quantities in the diet. The exposure was daily for most foods and drinks. If HCC depends on the combination of aflatoxins and HBV, the causes of hepatitis also take part in this analysis. HBV is a sexually transmitted disease or, in general, transmitted through body fluids. Any of its risk factors in Mozambique shall be seen as amplifiers for aflatoxin effects.

The last factor highly influencing the exposure is possibly the most relevant: the food insecurity. According to Casadei [13], the destruction of contaminated foods and feeds was highly discouraged just after the independence. There was a civil war compromising the production, political feuds certainly limiting trade between Mozambique and South Africa and Rhodesia (now Zambia and Zimbabwe), extreme cases of draught, among many other challenges for the new government. The food could not be destroyed because there was a shortage. Instead, it was usually cleaned, diluted in non-contaminated stocks or sent to refined oil factories out of hope to reduce contamination. The food insecurity remains, still conflicting with food safety issues. Indeed, eradicating hunger and poverty are arguably the hottest international topics and priorities for the United Nations (UN) [24,25].

The circumstances influencing aflatoxin intake in Mozambique form a complex network of challenges to be addressed, some natural and technical, and other social. The natural and technical just require some expertise, resources and technology. The social are more delicate and sensitive. How to effectively challenge people's habits and traditions? How to reduce production if there is hunger internally and international incentive to mitigate food insecurity? The **Figure 1** shows a summary of what was discussed in this section, with some modifications.

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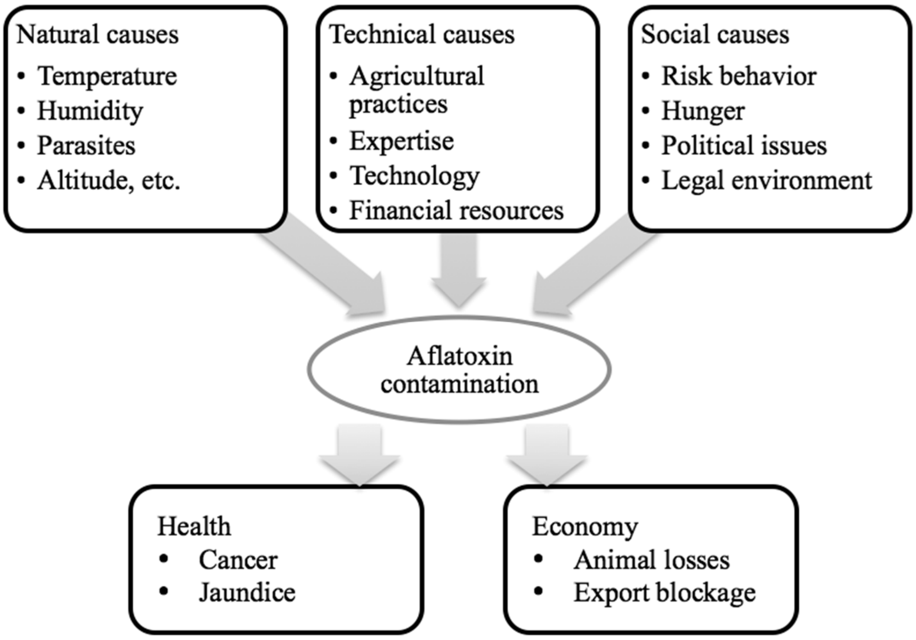

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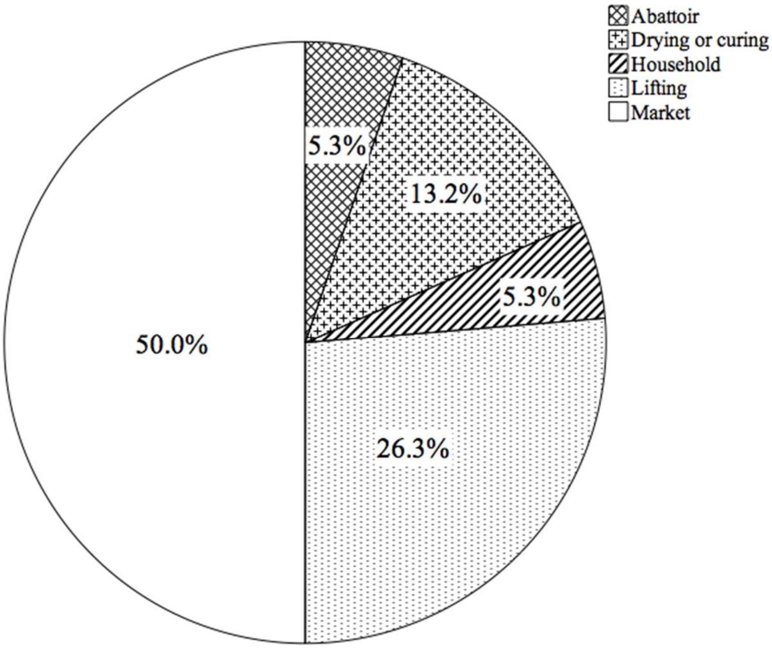
188 **Figure 1.** Causes and impact of aflatoxin contamination.

189 Where along the food chain have the aflatoxins have been found in Mozambique? The **Figure 2**
190 is a meta-analysis showing where most samples were taken from. Yet, it is important to know that
191 different samples were considered separately, even if collected at the same time for the same study.



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193 **Figure 2.** Pie chart showing at which step of the food chain the samples were found in most studies.
194 Based on Casadei [13], Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and
195 Purchase [23], van Wyk, Van der Merwe, Subrahmanyam and Boughton [4], Warth, Parich,
196 Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] and Sineque, Macuamule and Dos
197 Anjos [20].

198 The majority of the samples were purchased in farmers' markets and shops, probably because
199 at this stage the products are considered ready for domestic consumption. The samples from
200 abattoirs were also already approved for commercialization [20]. Furthermore, it is also easier to get

samples in this way, with no need of permission from some ethics committee if the researchers can afford the price. Other samples were collected during harvest, post-harvest, certainly in experimental fields. The samples from households shall be regarded as the ultimate evidence of exposure, though these were related with the medical studies by Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23], possibly outdated.

The quantity of samples contaminated seems to increase as they move from farm to market (**Error! Reference source not found.**). This is probably in part due to the pest management and the existence of natural enemies in the farm. Furthermore, the product is mixed during transportation and storage, increasing the odds of cross-contamination. However, the level of contamination per sample showed a different trend. It seemed to be the lowest the factory and market but abruptly increased in the households.

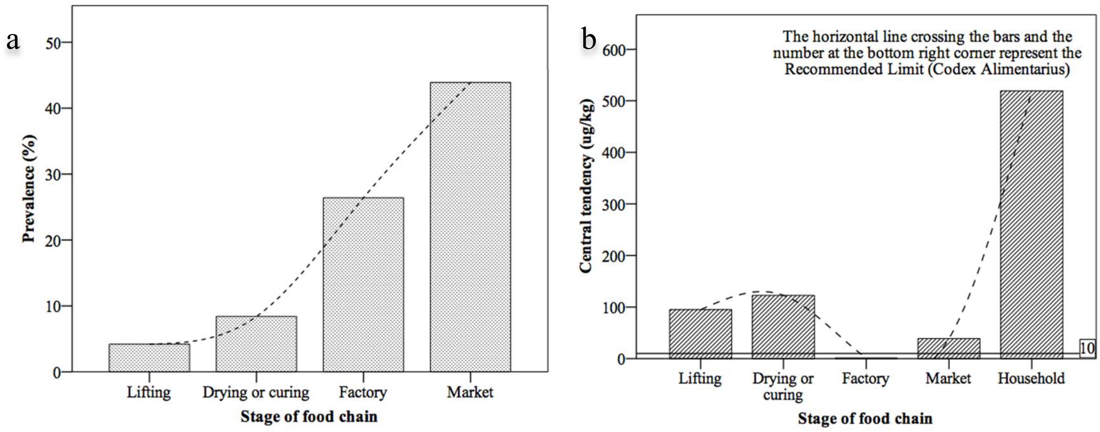

Figure 3. The prevalence (a) and level (b) of aflatoxin contamination in the major stages of the food production chain. Based on Casadei [13], Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23], van Wyk, Van der Merwe, Subrahmanyam and Boughton [4], Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] and Sineque, Macuamule and Dos Anjos [20].

The factory and market are expected to have lower mold infestation and aflatoxin levels in their commodities because they select and try to offer good quality products, as they are prone to be more consumer-driven. The households, however, frequently do not have the resources and sometimes level of concern to keep up with the high food safety standards as the companies. From these observations we can conclude that consumers have the most quantitatively and qualitatively contaminated foods and focus to control the aflatoxin should be at that end of the food production chain.

3. Geographic distribution of aflatoxins and related diseases

3.1. Geographic distribution

By the turkey X disease episode [26]. Brazil can be assumed as an early hotspot of aflatoxicosis. Ozturk [27] mentioned African countries such as Mozambique. Uganda. Kenya. Senegal. Swaziland. Nigeria, and Asian, such as China. Thailand and Philippines. Now, there is a wide literature from virtually the entire world [2,28], especially after the Kenyan incident and initiatives from IARC [29] and Codex Alimentarius Commission [30].

The **Figure 4** shows where aflatoxins were detected in Mozambique and in which commodities. Casadei [13] found aflatoxins in samples from different areas of Mozambique, though he did not specify which regions in his publication. Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] detected the toxins in samples from Inhambane. Augusto, Atehnkeng, Akello, Cotty and Bandyopadhyay [6] found in Manica. Zambézia. Tete and Nampula. Indeed, most of the recent studies were performed in Nampula [1,4,5].

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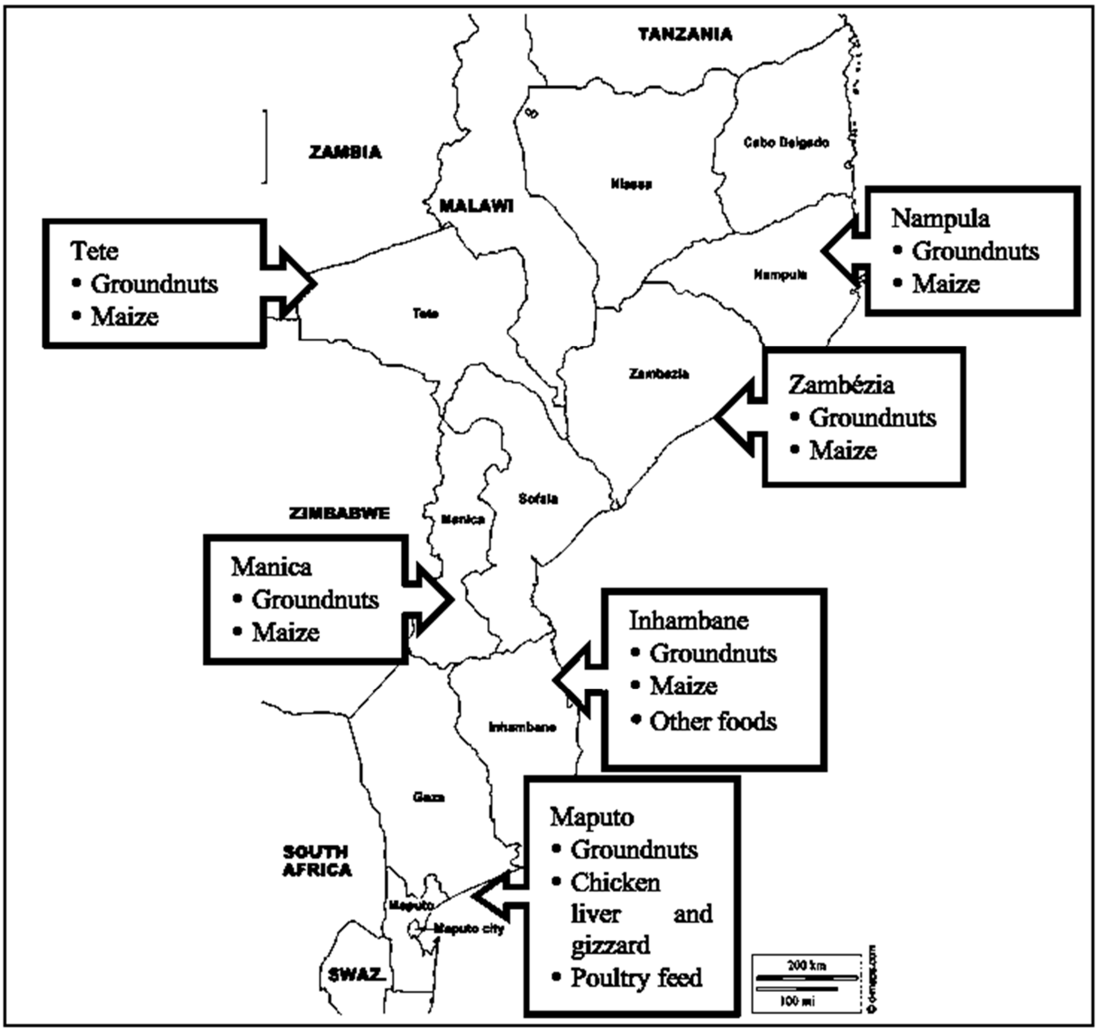

Figure 4. Mozambican provinces where aflatoxins have been detected and the products analyzed. Adapted from D-Maps [31] with data from Casadei [13], Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23], van Wyk, Van der Merwe, Subrahmanyam and Boughton [4], Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] and Sineque, Macuamule and Dos Anjos [20].

In this province, the highest toxin levels were found in Mugovola, Erati, Amendo and Murrupia districts. Some of the reasons behind the choice of Nampula are its potential as a major producer of groundnuts and other cash crops, its proximity to Nacala Port, a strategic point of contact with the Indian Ocean markets, and its importance as the country's third biggest urban concentration if Maputo and Matola are taken as a single urban continuum [32]. Finally, groundnut, feed and animal studies suggest the existence of contaminated samples in Maputo City and areas in the proximity [17,20,33]. There is a need to investigate in the remaining provinces: Gaza, Sofala, Cabo Delgado and Niassa. These areas are certainly also affected by aflatoxin contamination.

Regarding primary hepatic cancer. Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] said Mozambique had high incidence by world standards but not so if compared to other African and Asian countries. Still, the country held the highest prevalence Southern Africa [34], although it was decreasing [23], but it is difficult to compare the data from Mozambique with other countries as Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] stated there are many local discrepancies, even among neighboring districts. The found contaminated food in all districts from Inhambane province but Govuro and Vilanculuos, and Manhiça and Magude in Maputo province. Yet, the incidence is low considering the aflatoxin levels found locally. Actually, the toxin seems to simply boost the HBV power to cause HCC. Casadei [13] added that liver cancer affected annually, per 100.000 inhabitants, 21 people in

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rural areas and 17 in the cities. He said a survey in the Central Hospital of Maputo showed 28 people for each 100.000 inhabitants. Previously. Van Rensburg, *et al.* [35] described such levels as 100 times higher than the ones from western countries. Still, it does not explain at which extent it was influenced by aflatoxin intake.

Most authors preferred median as the main measurement of central tendency to profile aflatoxin levels, probably to minimize the effect of extreme values. However, other used means. It can impact the accuracy of the comparison between them, especially if the data are highly scattered. It would be wise to set a standard for this sort of measurement. In this case, a Wilcoxon signed rank test for related samples was performed, comparing the means and medians for the cases when both parameters are present. According to it, the differences between them were not significant ($p = 0.236$). Since there are more records with medians, they were all kept and the gaps were filled with average values. The mixed group of parameters was named central tendency.

It is possible to see more details about the spatial distribution of aflatoxin contamination (**Figure 5**). The highest prevalence was observed in Nacala, followed by Maputo City. Both are major commercial areas in Mozambique, where many products are imported and exported. If the products were purchased there, they might have come from the surrounding districts. Yet, it does not make the exposure any lower in these areas. Excluding Maputo, the top four areas with the highest prevalence are in Nampula province.

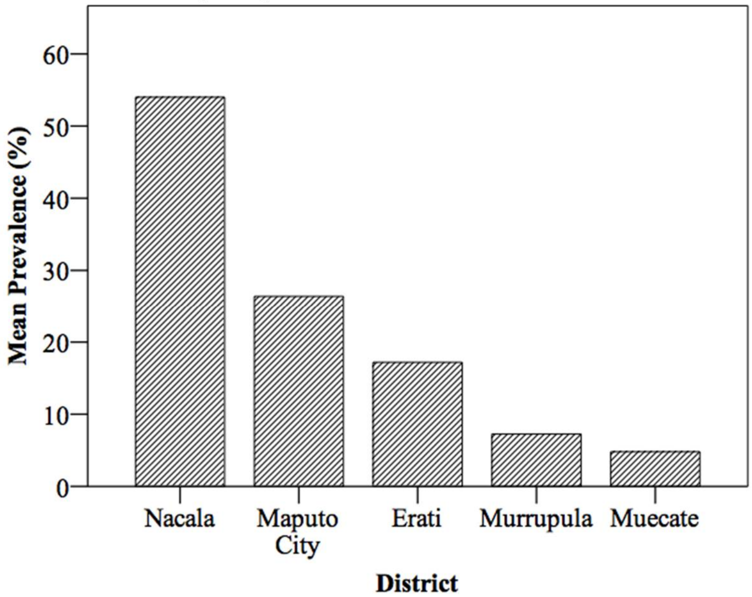

Figure 5. The districts with highest aflatoxin prevalence. Based on Casadei [13], Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23], van Wyk, Van der Merwe, Subrahmanyam and Boughton [4], Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] and Sineque, Macuamule and Dos Anjos [20].

But the situation might be more complex and this data biased. Nampula is also the province with the highest volume of recent research on the matter. Its data is more discriminated and detailed if compared to any other province. Also, one of the major features mentioned by Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] was the variation between neighboring areas. Thus, observations from few districts in a province might not be a good representation of what is happening in the others.

The districts with the highest aflatoxin levels (**Figure 6**) have records of extreme values if compared with the recommendation by the Codex Alimentarius. Inhambane showed the highest values, followed by districts of Nampula province. As it seems, the first province leads in amount of toxin per food and the second in how spatially spread they it is. Anyway, both seem to be hotspots of contamination, probably also with Zambézia [6].

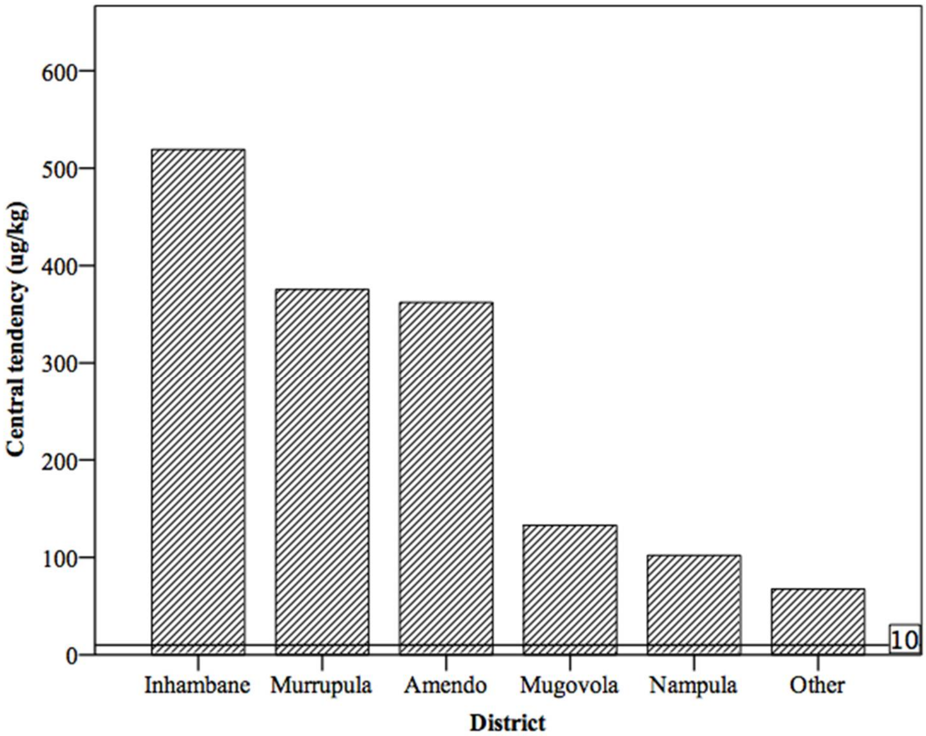

Figure 6. The districts with the highest aflatoxin levels in their foods. The horizontal line crossing the bars and the number at the right corner represent the maximum limit for peanuts (10 µg/kg) recommended by the Codex Alimentarius Commission [30]. Based on Casadei [13], Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23], van Wyk, Van der Merwe, Subrahmanyam and Boughton [4], Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] and Sineque, Macuamule and Dos Anjos [20].

3.2. Susceptible groups

Most research is focused in commercial foods. Thus, any person is susceptible to intoxication. The true level of exposure and impact of aflatoxin intake are not clear in Mozambique. The recent information covers very few foods, lacks information on the frequency and quantity consumed and the physiological effect in the population. For example, the exposure of the population in Inhambane should result in a higher incidence of HCC [23], in theory.

So, there must be some resistance to aflatoxin exposure. For this reason, it is safer to look at susceptible groups by profiling the known cases of HCC. Furthermore, this review will not cover acute aflatoxicosis as there is no academic information readily available about it in Mozambique.

Linsell and Peers [36] mentioned 101 cases of liver cancer in 576.782 individuals daily exposed to aflatoxin in their diets. This was the source from which Casadei [13] took the information for his book, then simplifying to make it more comprehensive. Most authors discussing the subject used directly or note the same article. Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] added by saying that this disorder was more prevalent among adolescents and young adults, particularly male. According to Shephard [34]. the incidence in males was 79.4 per 100.000. However, as the population aged, the sex ratio changed. Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] said this change is common in other parts of the world.

Again. the data above is old but it is the best available. The high incidence in male adolescent and young adults was probably due to their behavior and relatively high mobility if compared to the female counterparts. It makes them more susceptible to venereal diseases including hepatitis B. It also gives them less control over their diet, eating what is available wherever they go. As time goes

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by, they tend to settle, becoming more sedentary, responsible and creating families. This explains why the sex ratio of HCC changes, as they grow old.

4. Detection, prevention and control

4.1. Detection methods

Chromatography is the most frequently mentioned method for aflatoxin analysis in Mozambique since the early studies (**Figure 7**). Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] used thin-layer chromatography (TLC) when correlating toxin contamination in food with consumer's liver cancer.

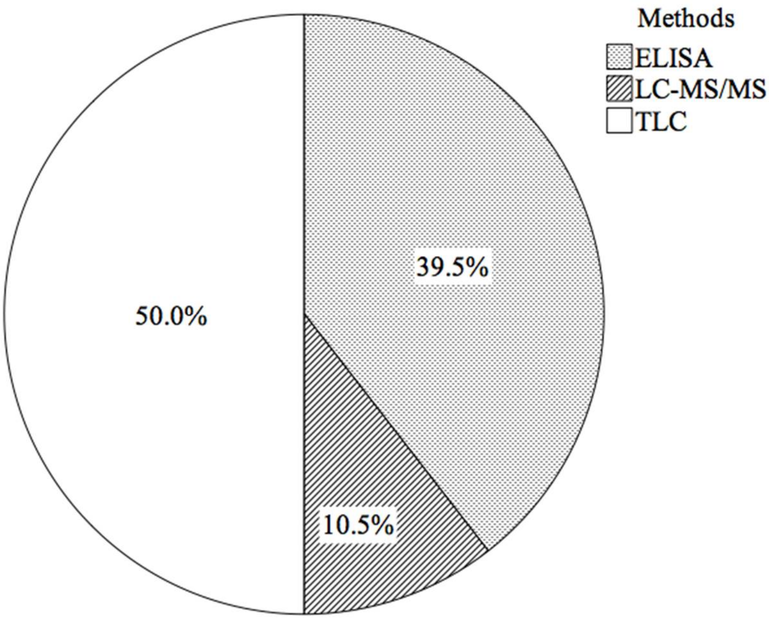

Figure 7. The most commonly used methods for aflatoxin analysis in Mozambique. Based on Casadei [13], Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23], van Wyk, Van der Merwe, Subrahmanyam and Boughton [4], Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] and Sineque, Macuamule and Dos Anjos [20].

Mondlane, Capece and Parruque [17] used the same method to detect AFB_1 in poultry feed. Essers and Nout [12] used high-performance thin-layer chromatography (HPTLC) for cassava but his results were inconclusive. possible due to scopoletin interference. Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] recurred to liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify mycotoxins in samples from Mozambique and Burkina-Faso Anjos, Ledoux, Rottinghaus and Chimonyo [19] analyzed feed for chicks through high-performance liquid chromatography (HPLC). Harmsen, Bremmer and Maria [3] recommend the latter method for the new laboratory of the Tertiary Polytechnic Institute of Manica.

The second most commonly used method was ELISA. Actually, this is probably the most common but not for academic purposes. It is very convenient for screening, especially when there is no need for quantitative measurements. Sineque, Macuamule and Dos Anjos [20] used this method to detect AFB_1 in chicken livers and gizzards. They recommended it as considerably rapid and economic technique for monitoring. Their suggestion was for feed and animal products but it is certainly good for many other types of commodities. However, they also said the method still required some improvements to guarantee more accurate results.

After using LC-MS/MS, Warth, Parich, Atehnkeng, Bandyopadhyay, Schuhmacher, Sulyok and Krska [1] praised the method, describing it as powerful because it can analyze up to 28 different mycotoxins in a single sample. This machine can be a good asset for Mozambique, because very little

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is known about other mycotoxin in its food or feed, but the authors could detect several of them in samples from Nampula.

All methods have strengths and weaknesses. They can be costly and most require top-of-the-art equipment and trained personnel to operate [3]. Yet, investing in them can pay an important part for the improvement of the public health.

4.2. Prevention

This topic is poorly explored in Mozambique, despites of its vital relevance. Yet, there are some recommendations to both minimize aflatoxin exposure and the risk of liver cancer. The few will be presented here but the best idea to future researchers and professionals in this country is to search for the high volume of worldwide literature because most techniques suggested everywhere else are likely to also work in Mozambique.

For groundnuts, Zuza, Mondjana, Muitia and Amane [5] recommend the proper post harvest management of groundnuts rather than delaying the harvesting time. This line of research was not new [37,38] but it was a good initiative to reproduce in the Mozambican context to see what would happen. Harmsen, Bremmer and Maria [3] commented on the storage techniques, suggesting jut bags rather then plastic. Their recommendation was not straight out of experiment; instead it was simply a commentary in a report about a new academic laboratory in Manica province. Yet, his expert opinion shall be accounted. There is some scholarly literature on the subject [39,40] and it is a good idea to study that in Mozambique.

Regarding to liver cancer, Van Rensburg, Cook-Mozaffari, Van Schalkwyk, Van der Watt, Vincent and Purchase [23] discussed on the dual impact from both aflatoxins and HBV. They said preventive measures for aflatoxin exposure should be integrated with hepatitis B vaccination to better alleviate the risk of cancer. However, if the measures were adopted, the results were never shared in public.

4.3. Prevention

In his paper, van Wyk, Van der Merwe, Subrahmanyam and Boughton [4] recommended Mozambican authorities to follow the steps from the international counterparts. So far, several professionals and institutions have directly or indirectly driven their efforts to improve public health and food safety over the years. There is still a lot to be done but at least such endeavors were in the right direction and can be taken as the foundation for very effective campaigns towards the reduction of aflatoxin contamination in Mozambique.

The Institute for Agricultural Research (IIAM) rehabilitated its laboratory for food analysis and initiated a general survey. It aimed to start a surveillance program for hepatic cancer in Mozambique Casadei [13]. Such task would target the all steps of the food production chain, from farms to markets. At that time, the common food processing steps, including selection of non-infested seeds, were reported to reduce the aflatoxin contamination by around 70%. However, there is no recent information on the matter or even if there were further surveys.

The International Institute of Tropical Agriculture has actively been working in several African countries to minimize aflatoxin contamination through bio-control [41]. The institution's strategy was to develop a pesticide based on endemic non-toxigenic strains of *Aspergillus* able to compete with the toxigenic counterparts, reducing considerably the population. The product was effective in Nigeria and Kenya [41], and recently it has been introduced in Zambia and Mozambique [14]. The project also includes a campaign to raise awareness and provide advice to the local policy makers.

Bentonite clay's adsorbent properties have been explored as an alternative aflatoxin control method for animals. Anjos, Ledoux, Rottinghaus and Chimonyo [19] found it to reduce poisonous effect of aflatoxins in poultry feedstuff. However, it probably needs further research because it was only partially effective. Once this method is fully developed, it can be easily adopted because the adsorbent is very abundant in the country.

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5. Recommendations

Knowledge is necessary for an effective aflatoxin control [14]. As there are limited resources for research, emerging economies like Mozambique can start by observing the progress in other places [4]. For example, many guidelines for farmers and other stakeholders are virtually applicable anywhere.

Regarding harvest, the time of crop's physiological maturity is ideal to ensure minimum contamination [5]. However, it helps but does not necessarily solve the problem. It is important to adopt an integrated pest management system. For example, further stages such as storage also require attention because *Aspergillus* keeps growing if the temperature and humidity allow [18].

Since some areas in Mozambique, such as Inhambane, Nampula e Zambézia are known hotspot areas, the current efforts for research and intervention should be more focused in this areas [6]. This is actually happening already in Nampula, but very little is said about the other provinces. Since Nampula and Zambézia are neighbors, it would be just a matter to extend the effort invested in the former to the latter.

It is necessary to evaluate the cost-benefits of each detection method prior to introduction in Mozambique. Harmsen, Bremmer and Maria [3] mentioned some skepticism from the authorities to purchase HPLC to the laboratory of soil analysis in the Tertiary Polytechnic Institute of Manica. They said similar equipment was being scarcely used in Nampula and it would be wise to invest in something more affordable such as ELISA. Indeed, Sineque, Macuamule and Dos Anjos [20] used ELISA effectively and recommended for routine screening. Still, they said the method had some limitations and their observations could not be conclusive without a more reliable method to validate their approach.

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