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Relationship Between Aflatoxins Occurrence in Brazil Nuts and the Good Management Practices

Maria L. Vinhote[1], Henrique dos S. Pereira[1], Ériton G. Rubem[1], Ariane M. Kluczkovski[2], Janaina Barroncas[2], Heloisa Barros[2], Eirie G. Vinhote[3] & David F. S. Guimarães[4]

[1] Center of Environmental Sciences, Federal University of Amazonas, Brazil

[2] Faculty of Pharmaceutical Sciences, Federal University of Amazonas, Brazil

[3] SEPROR-AM, Brazil

[4] ICMBIO, Brazil

Correspondence: Ariane Kluczkovski, Av. Gen. Rodrigo Otavio J Ramos, 6200, Manaus-Am, Brazil; E-mail: ariane@ufam.edu.br

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Abstract

Combining sustainable development with the conservation of the Amazon Forest is a challenge, and one of the strategies could be the strengthening of production chains, such as Brazil nuts. In this context, good practices can be applied to promote the sanitary quality of the seed, in which the presence of a carcinogenic contaminant, aflatoxin, is a continuous issue as it can cause economic embargoes on the product. So, the study aimed to analyse the relationship between several variables for 3 harvests that affected the production of aflatoxin. The results were statistically significant for all extrinsic variables and indicated that treatments with and without good management practices, and interaction between factors influenced the greater occurrence of aflatoxin. Moisture content, as an intrinsic variable to the food, indicated a significant association with Brazil nut contamination

Keywords: aflatoxin, *Bertholletia excelsa*, moisture content, water activity

1. Introduction

Brazil nuts are a non-timber product from the Amazon Forest, obtained through extractive collection using practices that are usually rudimentary, and which can compromise the sanitary quality of the product, create risks to human health and influence its market value. The origin of these risks may be directly related to environmental factors in the forest or arising from handling conditions in the manufacturing unit.

The pods (fruits) are normally crowded in Brazil nut trees and in direct contact with the soil, exposed to the high relative humidity (RH%) and temperature of the Amazon Forest. The rainwater ingress, mechanical damage or damage caused by insects to the seed shell can occur (Taniwaki et al., 2019). Furthermore, long storage periods are common and can affect sanitary quality. Therefore, the preventive control of variables that promote contamination by microorganisms or toxic agents is important as some species of fungi are producers of mycotoxins, such as aflatoxins (AFL) (Reis et al., 2022; Taniwaki et al., 2018). Frequent ingestion of foods containing mycotoxins for a prolonged period may favor the development of hepatocellular carcinoma or other liver diseases, and ingestion of foods with a high degree of contamination in a short period may have hepatotoxic effects, such as necrosis and fatty degeneration in humans and animals (Stoev, 2024). In addition to the gene expression of the fungus, in the production of mycotoxins, extrinsic and intrinsic variables to the food have been considered, such as RH%, ambient temperature and chemical composition of the food, especially the water content (Jallow et al., 2021). Mycotoxins are classified as carcinogenic, secondary metabolites of fungi, and their toxicity is influenced by the amount and duration of exposure, age, and nutritional status of the exposed individual, in addition to environmental factors (IARC, 2016). Human exposure to mycotoxins through contaminated food is a public health issue worldwide, but in Brazil nuts, contamination by AFL has been studied and unfortunately can cause export embargoes (Eissa & Sebaei, 2023; Essawet et al., 2017). Therefore, the adoption of good manufacturing practices (GMP) in the production chain can impact sanitary quality, which is influenced by variables inherent to the production process and food, and this includes management models in cooperatives/associations that collect and supply nuts to processing plants. processing. Although the extractive system of Brazil nut production is an economic activity for thousands of families in some locations in the Amazon, some stages of the socio-productive process favor contamination by AFL with consequent risks to consumer health and economic losses (Guarigata et al., 2017; Brose, 2016). These steps can affect the water content of the seed and favor an increase in the moisture content of the nut, for example. Therefore, preventive actions in the production process are essential for production systems to evaluate both the presence of contaminants and the origin and risks inherent to consumption. Among the preventive systems for the sanitary quality of food for Brazil nuts the GMP are activities still in the extractive stage to reduce the risks to aflatoxin-producing fungi (Kluczkovski et al., 2020). In this sense, we can also consider that in addition to collection, the storage and transportation stages that precede processing at the plant, must also prevent the quality and health of the product, and thus associations/cooperatives can also enter the global tree nut market, such as suppliers of raw materials that are safe for consumption. In this context, the aim of the work was to relate the adoption of GMP with AFL contamination in a region of a Brazil nut production in the state of Amazonas (Brazil) over 03 (three) harvests.

2. Method

The research was carried out in 08 (eight) communities along the Purus River and at the Brazil nut processing plant, located within the Sustainable Development Reserve (RDS) Piagaçu Purus (PP), the area surrounding the RDS - PP and Outside RDS - PP in the city of Beruri/AM (Figure 1). The focus group was formed by extractive producers who were directly involved in the collection and commercialization of Brazil nuts, who were interviewed with approval of the number ethics code CAAE 30657420.4.0000.5020. The interviewees provided data that indicated the performance of GMP, including whether they performed some actions before sending them to the processing plant, such as: scheduled collection, visual sorting of seeds, washing or drying and whether they applied storage with aeration. Data collection was conducted in 03 (three) consecutive harvests, from 2019 to 2021. Sampling began in May 2019, followed to January 2020 and concluded in January 2021.

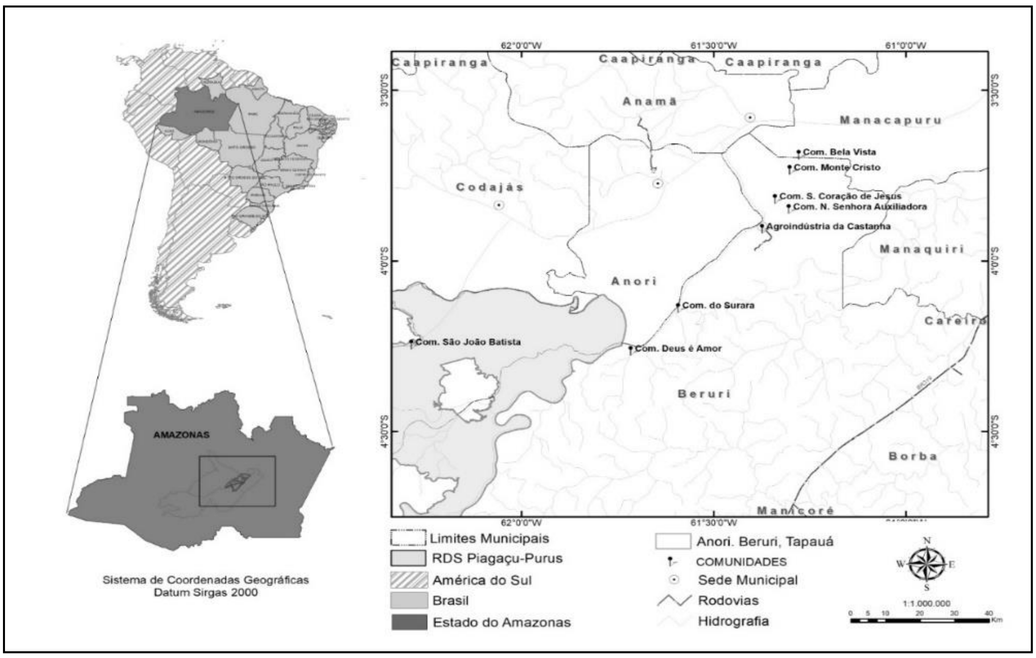

Figure 1. Sampling region in Brazil

2.1 Sampling Procedures

90 (ninety) samples of Brazil nuts were collected, 60 (sixty) were collected directly from the nut grove and/or warehouses in the communities listed in the research, 30 collected directly at the plant, 15 (fifteen) collected at the plant entry (beginning of the processing process) and 15 (fifteen) processed.

2.2 Assays

2.2.1 Moisture Content (mc %)

It was evaluated by AOAC (2016), using an electronic moisture balance (SHIMADZU, MOC-120H®, Kyoto, Japan) and an infrared dryer, drying approximately 1g of the sample at a temperature of 105 °C until all moisture was lost. All analyses were performed in triplicate.

2.2.2 Water Activity (Aw)

The Aw content was checked with an Aqualab benchtop meter series 4TE (DECAGON®) at room temperature (25°C) and used the dew point method. All analyses were performed in triplicate.

2.2.3 Aflatoxins

AFL (B1+B2+G1+G2) were quantified by liquid chromatography (HPLC), according to the AOAC Official Method (AOAC, 2016). In 50 g of sample, AFL were extracted with 100 mL of acetonitrile: water solution (90:10 v/v), stirred at high speed for 5 minutes with subsequent filtering with filter paper. Then, 3mL of the filtrate was transferred to a 10mL culture tube with application by MYCOSEP 226 cleaning column (Romer Labs) for purification. The resulting solutions were applied to HPLC (mobile phase - acetonitrile, methanol and ultrapure water 1:1:4), Waters X-Terra column, 150 x 4.6 mm, flow of 1.0 mL.min-1 eluting in mode isocratic, with fluorescence detector: excitation λ- 360 nm and emission λ- 440 nm; injection volume of 50μL; running time 20 min. AFL standards B_1, B_2, G_1 and G_2 (Sigma Aldrich) were used and the limit of detection (LOD) and limit of quantification (LOQ) for each toxin (AFB_1/AFB_2/AFG_1/AFG_2) were 0.136/0.136/0.250/0.250 and 0.410/0.410/0.750/0.750 μg/kg, respectively. The LOD method was defined by 3 times the signal-to-noise ratio and the LOQ by 6 times the signal-to-noise ratio. 5 points were used to construct an analytical curve to obtain the correlation coefficient (R) values for LOD and LOQ. Each point corresponded to an average of five injections of each extract. The recoveries for each AFL (AFB_1, AFB_2, AFG_1, AFG_2) were 94.5, 73.5, 97.8, and 99.1%, respectively.

2.2.4 Statistical Analysis

For statistical analysis, the software Past – Paleontological Statistics (Hammer et al., 2001) was used and for data processing, processed and fresh Brazil nut samples were tabulated, processed, and analyzed using regression multiple between the variables "Total Aflatoxin x Aw x mc%". With tow-way analysis of variance (bidirectional), the relationship between the following variables/treatments was analyzed: aflatoxin x with and without management x year of collection of samples.

3. Results

Table 1 indicates that there is a relationship between AFL content and mc%. It is also observed that there is no linear relationship, given that "p" was not significant for Aw. Therefore, in these samples, Aw does not seem to explain AFL contamination, since Aw only explains 0.8% of AFL contamination. However, mc% is significant, so it is possible to say that mc% appears to be a good predictor for the level of AFL contamination with 17% of the occurrence explained by mc% variation. Most samples had a mc% < 30%, which corroborates information from Botelho et al. (2019) that raw seeds from the forest, showed level of 38%.

Table 1. Relationship between mc% and Aw by regression analysis

	Coefic.	S	t	p	r^2
Constant	0.14672	0.64134	0.22877	0.81959	
Aw	-0.40653	0.98885	-0.41112	0.682	0.080981
Mc %	0.062421	0.020289	3.0766	0.002799	0.16955

Although Brazilian legislation uses a limit of up to 15% of mc% for processed seeds (Brasil, 2010), the Aw recommended limit by Europe is < 0.70. (CAC, 2010). Note that as mc% increases, there is a tendency for the AFL content to increase and this may be influenced by the tropical environment of the seed production chain, in which there is RH% (>75%) associated with the temperature (> 26ºC) as important factors in the production of AFL, cited by other authors. The storage time cannot be considered variable, as the samples came from different communities with different drying or storage times. In this context, Calderari et al. (2013) indicate that the seed drying process within 10 days post-harvest is important, as it can reduce AFL production by up to 98%. As Aw was not significant and does not explain AFL contamination, an invariable regression was carried out (table 2) where only the variables "mc% and AFL" were considered, and the results confirm that the variable that best explains the levels of AFL was the mc%.

Table 2. Regression analysis for mc % x AFL

	SS	gL	MS	F	p
Regression	37.791	1	37.791	17.966	5.53E-05
Residual	185.1	88	2.1034		
Total SS	222.89				

The relationship between the variables/treatments: AFL x with and without GMP x crop, was analysed with tow-way analysis of variance (bidirectional). For all variables, the results were statistically significant, both for the variables/treatments with and without GMP, as well as for harvest and interaction, therefore, they indicate that treatments with and without GMP, harvest and interaction influence the levels of AFL contamination. Table 3 shows both the harvest factor and the processing factor were significant. However, as the interaction was also significant, this indicates that depending on the harvest there may not have been differences between the levels of contamination between samples with and without GMP.

Table 3. Fixed effects Two-way ANOVA: AFL x Harvest x GMP

	Sum of sqrs	df	Mean square	F	p (same)
Processing:	13.8966	1	13.8966	7.578	0.007237
Harvest:	25.9421	2	12.9711	7.074	0.001448
Interaction:	28.8954	2	14.4477	7.879	0.000731
Within:	154.032	84	1.83371		
Total:	222.766	89			

In 2021, the contamination of unmanaged samples was greater than that observed in managed samples, therefore, with a statistically significant difference. That year, even without GMP samples had a higher average contamination value than in 2019 and 2020 and this difference was statistically significant. When comparing only the years 2019 and 2020, there were no significant differences between the levels of AFL.

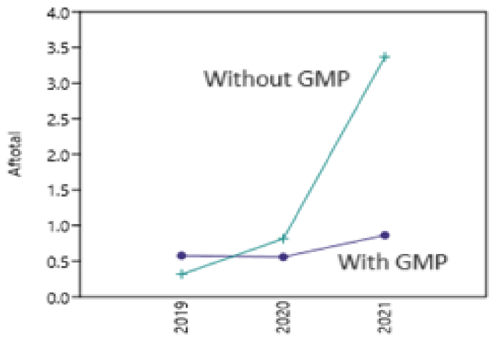

Figure 2. AFL in different Harvest in samples with and without GMP

The adoption of GMP (Pre-harvest and Post-harvest) of Brazil nuts can favor sanitary quality, but one of the biggest challenges in the exploitation of Brazil nuts is to build and effectively implement technical guidelines for GMP in all the segments prior to the industrial processing of Brazil nuts. Such guidelines can enable the adoption of a minimum protocol of guidelines that promote sanitary quality and sustainable management of extractive activities, respecting the environment, culture and dynamics of the populations involved. Álvares et al. (2012) mention that GMP involves mechanical, industrial, and artisanal processes. Even though it is a manual activity, collecting seeds involves planning, in which the extractor cannot leave the pods in the ground, with long exposure and which can rot. Another important activity in the process is the storage of these pods and seeds, as their long period of residence in the soil increases the possibility of fungal proliferation. When the managed samples are compared with the unmanaged ones (Figure 2) in the different harvests, it is observed that in the managed samples there is little variation in AFL contamination. The variation between years is only observed in samples without GMP. In 2019, even unhandled samples demonstrated less contamination by AFL. In 2020, there was a reversal in the variation between sample contamination levels, however, no statistically significant difference was observed. Apparently, for the years 2019 and 2020, having or not having GMP does not seem to have influenced contamination. However, in 2021, a greater statically significant difference was observed. Generally, in years of low production, such as 2021, seed growers do not select to avoid losing volume, they collect older, damaged and/or contaminated seeds from the previous harvest. In years of abundance, fresh seeds are harvested, and with volume, it is possible to select before selling to the buyer. Although seeds without GMP from 2021 indicate a higher AFL content than other analyzed samples, the levels found are still within the parameters permitted in the country and Europe. In Brazil, the maximum accepted levels of AFL for Brazil nuts are 10µg/kg for shelled almonds and 15µg/kg with shell for subsequent processing (Brasil, 2022). the AFL variables and samples with and without GMP. There are several samples handled outside the general distribution pattern, that is, outlier samples, therefore, they are more contaminated than most samples. When separated by harvest, in 2021, there was a higher level of contamination, and there is a wider dispersion of data and higher values. Therefore, in 2021 there was a different behaviour compared to the other 02 (two) years. Figure 3 shows Aw by crop, the crop x management interaction was not significant. The difference between managed and unmanaged samples was highly significant. Regardless of the harvest, Aw was always higher in unmanaged samples, on average.

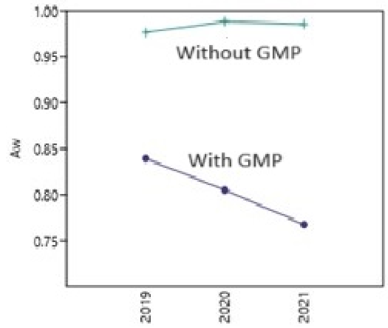

Figure 3. Aw in different harvests in samples with and without GMP

Figure 4 shows the average of mc% and the samples with and without handling. it was found that the mc% of the samples without GMP is different than that of the samples with GMP. In previous analyzes it was observed that mc% is associated with contamination, therefore, it is observed that the average mc% of samples without GMP differs statistically from the average of samples with GMP.

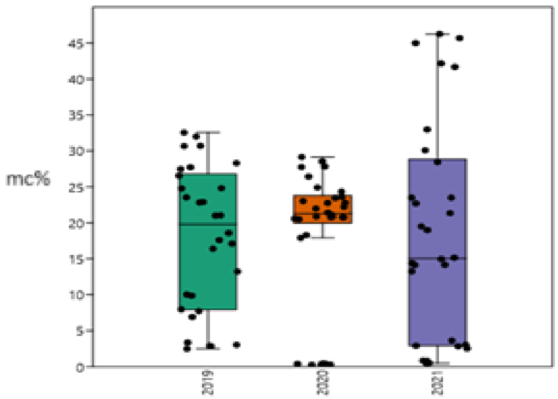

Figure 4. Mc% in samples with and without GMP

Regarding the mc% of the samples in relation to the year, it is observed that the distribution of the samples is very different. The 2020 samples apparently are more homogeneous. Following the trend of previous years, it is observed that in samples without GMP, the levels are higher, however, it is possible to verify that in 2021 there is a tendency for mc% to vary. Also in 2020, there was uniformity and little dispersion of data. Therefore, this pattern is repeated in the years 2019, 2020 and 2021, and in all analyses carried out, they demonstrate that unmanaged samples are wetter. Therefore, it is explained that AFL contamination appears to be directly related to mc% and it was always higher in unhandled samples. Therefore, it is inferred that GMP adopted in the Brazil nut production can corroborate the sanitary quality of the raw material. In figure 5, correlating AFL with the sampling points, it is observed that there are no contaminated samples at collection point 3, a result arising from the processing and processing of seeds at the plant. However, at point 1, there are contaminated samples indicating that contamination may begin in the forest.

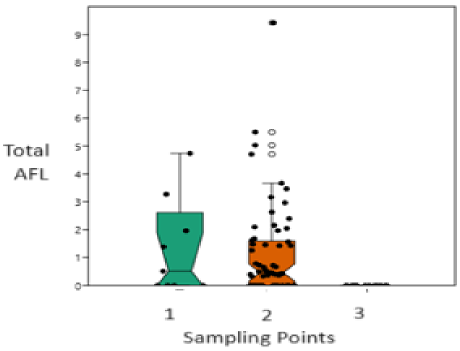

Figure 5. AFL in different sampling points

4. Conclusions

The occurrence of AFL in Brazil nuts obtained under GMP was compared with samples without GMP. Considering the results, the Aw content does not seem to explain AFL contamination, however, mc% seems to be a good predictor for the level of AFL contamination of seeds. In 2021 it was observed that the contamination levels of samples without handling were higher than those obtained with handling. The 2021 harvest was considered bad (less productive). In years of low production, Brazil nut growers stop collecting the seeds to avoid losing volume, or collecting old, damaged, or contaminated seeds. It is concluded that AFL contamination is directly related to the mc% and was higher in samples without GMP. Therefore, it is inferred that GMP adopted in the Brazil nut production can contribute to the quality of the product.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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