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Aflatoxins: A Brief Review of their Chemical Properties, Toxicological Effects and Control Measures

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Abstract

Aflatoxins are toxic secondary metabolites produced by the fungi of the genus *Aspergillus*. These substances cause food poisoning with clinical manifestations that vary according to the time of exposure and concentration of the dose ingested, representing a serious public health problem for compromising the food security, also causing considerable economic losses both in the production of stocked vegetable foods, as well as in the livestock contaminated with these substances through the feed. Therefore, this literature review aims to introduce some aspects related to the contamination of food by the fungi of the genus *Aspergillus*, the chemical and toxicological properties of the aflatoxins, as well as the strategies of control to avoid them in food.

1. Introduction

The provision of food containing nutrients and devoid of food hazards is an essential factor for the establishment and maintenance of the health and quality of life of the population. However, often in the food production, there are critical points during the processes of raw matter processing, packaging, transport, and storage that can favor the food contamination by physical, chemical, or biological hazards, highlighting that the microbial contamination can be mitigated or enhanced by factors intrinsic to the food, or pertinent to the conditions of the environment of the processes mentioned above. **(Vågsholm *et al*. 2020)**.

In this context, many foods of plant origin are susceptible to microbial contamination by fungi that produce toxic substances called mycotoxins. And this situation represents an economic problem due to the deterioration of foods by the fungal growth and release of metabolites that turn their use in the human and animal nutrition unfeasible, generating economic losses reinforced by the sanitary barriers imposed on the international market; and also a public health concern due to the harmful effects that these substances can cause in the long term (**Pereira; Santos, 2011; Baquião, 2012; Sacramento,2016; Sousa, 2018**).

Therefore, this work aims to expose, through a literature review, the biological aspects of the genus *Aspergillus*, the chemical and toxicological properties of the main group of mycotoxins produced by this genus - the aflatoxins-, and also expose the main strategies of control and prevention of aflatoxins in foods.

2. Material and methods

For the development of this work, we conducted an online literary survey with the terms aflatoxin and *Aspergillus* that resulted in the obtaining of 70 works, being 28 selected, including 12 articles, 5 thesis, 2 monographs, and 1 book. As selection criteria, we adopted the pertinence to the theme and the economic viability regarding the strategies of control of aflatoxin in foods.

The results obtained are presented in an explanatory way, according to the methodology described by **Cooper (1988),** presenting a focus on the analysis of the literature about the chemical properties of the aflatoxins, their toxicological effects, and control measures, aiming to raise data that provide subsidies for future works of applied nature. Therefore, this review is directed to the public of the agrarian, biological, and health sciences.

3. Results and discussion

The genus *Aspergillus*

The genus *Aspergillus* belongs to the group of the Hyphomycetes, which is characterized by the formation of specialized hyphae and conidia with variable shapes and architecture. It is a genus strongly associated with the deterioration of dry foods that are hardly attacked by other microorganisms that require higher water activity, also deteriorating foods stored in improper conditions of drying and storage (**Santos, 2008**). This fungic genus can develop and produce toxic secondary metabolites called mycotoxins, which can be contained inside the spores and

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mycelia, and then be released into the food contaminated by the fungi of this genus (**KwiatkomoskI; Alves, 2007**).

Among the mycotoxins produced by the genus *Aspergillus,* it is possible to mention the aflatoxins, penicillic acid, citrinine, sterigmatocystin, ochratoxin, and patulin, which are substances that, depending on the dose, can cause acute, subacute, or chronic intoxications, mainly affecting the liver, and also possessing the potential to cause or induce cancer. Such toxins are produced mainly by the species *Aspergillus flavus*, *Aspergillus melleus*, *Aspergillus niveus*, *Aspergillus rugulosus*, *Aspergillus ochraceus*, and *Aspergillus clavatus*, respectively; emphasizing that the same toxin can be also produced by more than one species of *Aspergillus* (**Diniz, 2002**).

The identification of this genus is easily accomplished by analyzing their main morphological features, which are the formation of colonies with white mycelium, and spores that may present a coloration that varies from green to yellow, gray, brown, or black; possessing at the microscopical level hyaline hyphae with septa (**Morais, 2018**), and the presence of conidiophores that generally present an enlarged structure called vesicle in which a layer of sterile cells called metula are present giving support to the cells that produce the spores, which can be arranged in rows (phialides) over the *Aspergillus* "head" covering it totally or just at the top (**Francisco, 2017**).

The classic microbiological identification for this genus and its species is carried out through morphological analysis and the growth profile of the fungus in the media recommended by the literature, such as CYA (Agar Czapek Yeast Extract - K_2HPO_4 1.0g, Czapek Concentrate 10 mL, 1 mL metallic solution, Yeast Extract 5.0g, Agar 15.0g, Sucrose 30.0 g, and distilled water 1000 mL), MEA (Agar Malt extract - Malt extract 20.0g, Peptone 1.0 g, Glucose 20.0g, Agar 20 and distilled water 1000 mL), and the BDA medium (Potato Dextrose Agar - Potato infusion 4.0g, Dextrose 20.0g, Agar 15g, and 1000 mL of water) with an incubation period of approximately 7 days at the appropriate temperature (**Monteiro, 2012; Sia, 2012; Gava, 2002**).

Since the great majority of the fungi are mesophiles, with an optimal growth temperature between 20 ° C and 30 ° C, the genus *Aspergillus* can be easily distinguished from other molds because it is thermotolerant, managing to germinate their spores at temperatures above 37 ° C, presenting an optimal temperature ranging from 30°C to 40 ° C (**Poester *et al*., 2015; Shabo, 2014**).

Although its morphological characteristics have great value in the classification and taxonomy, the genus can also be identified by analyzing the profile of the secondary metabolites produced, and molecular biology techniques that allow the obtaining of results in a short time with great precision (**Monteiro, 2012**).

Aflatoxins

Mycotoxins are fungal products that are dependent on the occurrence of a consecutive series of reactions catalyzed by enzymes that lead to their production, being accepted that their biosynthesis is the result of accumulations of metabolic intermediaries in the fungi primary metabolism, which in order to maintain the primary pathways operating, performs deviations of the intermediates in excess for the production of mycotoxins (**Dias, 2018**).

Being the aflatoxins mycotoxins produced by the genus *Aspergillus* usually detected in foods containing glucose, sucrose, and fatty acids at temperatures between 25 and 30 ° C, mostly in foods from tropical countries due to the high temperature, such as peanuts, rice, beans, corn, barley, and almonds such as the Brazilian nuts (**Dias, 2018; Lopes, 2012 Gonçalves *et.al*, 2017**).

The aflatoxins are classified in groups designated by the letters B, G, and M, in which the letter B is derived from the Blue fluorescence emitted by the substances of the group B when exposed to UV radiation, the letter G is derived from the Green fluorescence that the aflatoxins of the group G emit when exposed to UV radiation; and the letter M designates the aflatoxins mainly found in the Milk (**Baquião, 2012**).

The most common aflatoxins in foods are the aflatoxins B1, B2, G1, and G2, where those of the group of B are produced mainly by the fungi of the species *A. flavus* and *A. parasiticus*, while those of the group G are produced by the species *A. parasiticus*, being the aflatoxins M1 and M2 derived from the animal metabolism over the toxins of the group B, and they affect humans through the consumption of milk or meat contaminated by the urine of animals exposed to the fungal toxins of the group B, or from animals whose food was made with raw matter contaminated by B aflatoxins of the group (**Moreira, 2018**).

Generally, when isolated, the aflatoxins are crystalline, colorless or yellowish, insoluble in non-polar solvents (except chloroform), and moderately soluble in polar solvents such as methanol, and dimethyl sulfoxide, presenting a solubility of approximately 10-20 μg. mL^{-1} in water; they also are substances extremely resistant to the heat (thermostable) that start their degradation process at temperatures in the order of 220 ºC (**Pierezan, 2013; Cruz, 2010**).

The aflatoxins can be differentiated by 1) their low molecular weight, in which the aflatoxin B1 has a molecular mass of 312 g. mol^{-1}, the aflatoxin B2 has a molecular weight of 314 g. mol^{-1}, the aflatoxins G1 and M1 328 g. mol^{-1}, and the aflatoxins G2 and M2 have a molecular weight of 330 g.mol^{-1}; and 2) by their melting points, in which the aflatoxin B1 has a melting point of 269 ºC, the aflatoxin B2 has a melting point in values ranging between 286-289 ºC, the aflatoxin G1 changes from the solid to the gaseous state at temperatures between 244-246 ºC, aflatoxin G2 at temperatures between 237-240 ºC, aflatoxin M1 at values of 299 ºC), and the aflatoxin M2 melts at a temperature of 293 ºC (**Pierezan, 2013**).

In structural terms, it can be noted that the difference between the aflatoxins B1 and B2 is due to a double bond present in the position C15, differentiating both from the aflatoxins belonging to group G by the presence of a second cyclic ester between C3 and C4, with a double bond at the position C15 in the aflatoxin G1, and its absence in the aflatoxin G2 (**Bordini *et al*., 2013**); the same difference is also found in the toxins of the group M concerning the C15 position, differentiating them from the others by the hydroxylation after the hepatic metabolism in the C14 position. Figure 1 shows the structures of aflatoxins B1, B2, G1, G2, M1, and M2.

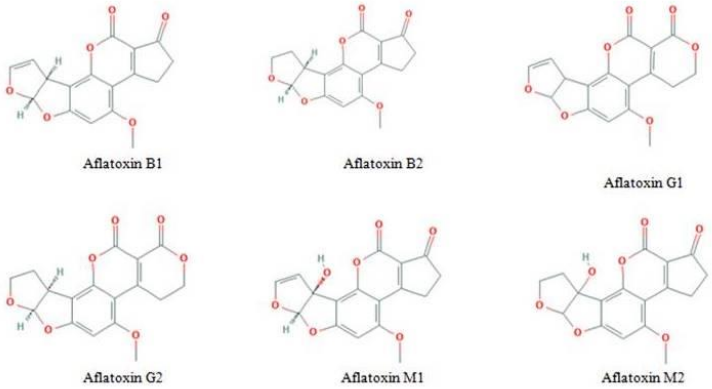

Figure 1. Molecular structure of the aflatoxins. **PubChem (2020)**

Aflatoxins can be detected by various analytical methods, such as gas chromatography (GC), high-performance liquid

chromatography (HPLC), thin-layer chromatography (CCD), and ELISA (**Baquião, 2012**).

Mycotoxicosis

The poisoning caused by mycotoxins is called mycotoxicosis, and their main effects on the human and animal organism are the production of injuries to the liver, kidney, system nervous system, and behavioral changes, as well as a high potential, to induce cancer. Highlighting that the mycotoxins present as the main forms of access to the organism the oral, inhalation, and topical routes, where the oral and inhalation routes are the commonest in cases of intoxication, respectively through the consumption of contaminated food, or by inhaling spores. (**Gonçalves *et al*., 2017; Pereira; Santos, 2011**).

The mycotoxicoses caused by aflatoxins receive the name of aflatoxicosis, and they tend to present in humans a degree of severity that depends on variables such as age, nutritional status, pre-existing disease, dose, and the manifestation of the symptoms is strongly dependent on the dosage and time of exposure, where high doses cause acute intoxication with the occurrence of damage in the liver, changes in the digestion, absorption of nutrients, edema, and hemorrhages that can happen along with a high chance of evolution to death in a short time; being the development of hepatic cancer common in cases of chronic intoxications due to low exposure for a long time. (**Pereira; Santos, 2011; Vitorino, 2011; Rosmaninho, *et al*., 2001**).

In animals, the main symptoms are reduced growth, motor, and behavioral changes, as well as reduced or loss of the reproductive capacity with progression to death, and it is often difficult to identify this type of intoxications because the adverse effects tend to be sub-clinical (**Gonçalves *et al.*, 2017; Vitorino, 2011; Rosmaninho, *et al*., 2001**).

Considering the effects of aflatoxins over the animal production, **Pierezan *et al.* (2012)** report that the ingestion of aflatoxin B1 in the feeding and water of calves in concentrations of 1.250 ppb, 2.500 ppb, and 5.000 ppb resulted in diarrhea and profound weight loss a few days after the beginning of the administration. And **Michellin (2014)** reports that the administration of aflatoxin B1 to fishes of the genus Astyanax in the feed at concentrations of 10 µg.kg-1, 20 µg.kg-1, and 50 µg.kg-1 can directly affect humans through the food route because this toxin accumulates in the muscle tissue and liver of the fishes.

In other animals, **Oliveira *et al.* (1997)** report the development of liver cancer after prolonged administration of doses of aflatoxin B1 ranging between 15-1000 µg.kg-1 to rats, while **Guterres *et al.* (2017)** report that prolonged exposure of dogs to the same aflatoxin through the feeding in concentrations between 100-300 ppb resulted in vomiting, diarrhea, anorexia, melena, coagulopathies, jaundice, and sudden death.

Such studies demonstrate the severity of the effects of the aflatoxins on food as a public health problem, and its potential to generate significant economic losses in animal production. Therefore, it reinforces the importance of the theme and development of research seeking strategies to fight the fungal contamination in food, as well as the contamination by aflatoxins.

Control methods

The foods most susceptible to contamination by mycotoxigenic fungi are those that present favorable conditions for this to occur, where the intrinsic and extrinsic factors inherent to the food influence the development of these microorganisms, emphasizing that all they act in a combined manner (**Pereira et al., 2002**). Highlighting that the fungal contamination can occur during the harvesting, transportation, and storage, mainly due to the lack of proper hygienic-sanitary conditions that consequently favors the contamination by mycotoxins after fungal development (**Schabo, 2014**). What makes relevant the application of strategies of control to avoid this problem through preventive or corrective (**Sassi, 2015; Lopes, 2012**).

The preventive forms of control can be applied in the pre-harvest and the post-harvest period, wherein the pre-harvest, actions such as the correct irrigation, protection against insects, the supply of nutrients to the plants, biological control of diseases, crop rotation, elimination of residues can reduce the susceptibility of the plants against the fungal infection, and improve the quality and safety of the harvest. While in the post-harvest, measures such as harvesting in the dry weather, the removal of the damaged grains, adequate drying conditions (below 10%), proper temperature and humidity control during the storage, pest control, and cleaning of the processing and storage facilities can avoid the contamination of the food by fungi in general, and consequently prevent the contamination by mycotoxins (**Lopes, 2012**).

While the corrective actions employ methods whose purpose is to degrade the mycotoxins in the food and reduce their concentration to an acceptable level through approaches that use physical, chemical, or biological agents.

In the physical approaches, the foods are exposed to physical agents such as the sunlight, high temperatures in the process of roasting, wet grinding, and radiation as the ultraviolent, and the gamma radiation, highlighting that this last physical agent is still under study. While in the chemical methods, the foods are exposed to substances such as ammonia, hydrogen peroxide, sodium bicarbonate, ozone; and the biological approaches can be exemplified by the use of lactic acid bacteria fermentation that reduces the levels of aflatoxin in some foods, and the use of adsorbents during the livestock production that prevents the absorption of mycotoxins by the animals, therefore reducing their toxicity (**Lopes, 2012; Moreira *et al.*, 2018**).

4. Conclusion

The aflatoxins constitute a public health problem, mainly because they can affect many people in the population, and their signs and symptoms are often confused with many diseases, also compromising the production of livestock and food, causing losses at different scales.

In this review, different approaches for the detection, prevention, and correction of mycotoxin contamination in foods were exposed, being their cost of application variable depending on the size of the production and the financial resources of the producer. Where in general, the detection of mycotoxigenic fungi is the most viable measure because it directly indicates the presence of fungi in the production and also favors the detection of critical points where the hygienic-sanitary measures must be reinforced.

However, in cases where the fungal contamination has already happened, to avoid large losses of the production, the adoption of corrective measures is desired, but their costs may represent a limitation for producers who don't have the financial resources available to invest in the mitigation of the chemical hazard in the food.

Therefore, this work strongly suggests the adoption of preventive methods to avoid the deterioration of foods by fungi, as well as the contamination by mycotoxins. And in this context, it is also important that the academic community direct efforts to seek means to lower the costs of the corrective methods, making them more affordable for different scales of production to assure the safety of the food reaching the final consumer.

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