

Occurrence of mycotoxin-producing fungi in coffee beans marketed in Goiás

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Jacqueline Farias Rodrigues de Lima

Heloísa Gabriel Falcão

Simone Silva Machado

Keyla de Oliveira Ribeiro Miguel

Angel José Vieira Blanco

ABSTRACT

Fungi constitute a heterogeneous group of microorganisms with diverse ecological activity. Many fungi are useful to mankind, while others are quite harmful. Besides being spoilage organisms, some genera of fungi are toxigenic and produce dangerous secondary metabolites called mycotoxins. Mycotoxins are low molecular weight, thermo-resistant compounds that can be found in various components of human food. Coffee is one of the foodstuffs with the highest incidence of toxigenic fungi, and the presence of these organisms can cause serious health problems in those who consume the beverage prepared. The absence of good practices

along the production chain facilitates the development of these fungi, especially species of *Aspergillus*, *Fusarium*, and *Penicillium*. Some studies show that the mycotoxins produced by these fungi are not eliminated during the process of roasting the beans or after boiling the coffee, which shows the importance of detecting their presence, in a preventive way, before the roasting stage. The present work evaluated the occurrence of mycotoxin-producing fungi in coffee beans, commercialized in the raw form, dry, unshelled, and ready to be roasted, of five different brands commercialized in Goiás. Coffee beans were inoculated in a BDA medium and after the formation of colonies, microcultures were performed on slides for further identification. The results showed high levels of fungal contamination, with a predominance of *Aspergillus niger*, a species commonly found in soils, which produces one of the most potent mycotoxins known, ochratoxin A.

Keywords: Contamination, Fungal diversity, *Coffea arabica*, *Aspergillus*, Food.

1 INTRODUCTION

Originating in the mountains of Kaffa, Ethiopia (SOUZA, 2006), coffee was introduced to Brazil in 1727 from French Guiana, and due to favorable climatic conditions, its cultivation spread throughout the country (BRASIL, 2018). Plant of shrubby size or arboreal, with a woody stem, and with a perennial life cycle, the coffee tree belongs to the Rubiaceae family and has about 500 genera and more than 6000 species, *Coffea arabica* and *Coffea canephora* the main cultivated and commercialized species, which give rise to the drinks Arabica coffee and robusta coffee, respectively (CARVALHO et al., 2017).

Coffee is one of the main agricultural products in the world, moving billions of dollars every year (BRASIL, 2022) and employing about 500 million people (REZENDE et al., 2013). It is in the group of the most consumed beverages on the planet, mainly due to its stimulating chemical nature and its refined

aroma and flavor. Such attributes value the café as a drink and contribute to an increasing number of connoisseurs.

In addition to its stimulating effect, associated with caffeine, it is known that coffee has antioxidant properties thanks to chemical elements, such as chlorogenic acids, existing in its beans (ALVES; COUPLE; OLIVEIRA, 2009). Americans are the largest consumers of coffee in the world, followed by Brazilians who occupy the second position in this classification (BRASIL, 2018). Perhaps this is why the United States and Brazil, in this order, are the two main countries in several scientific publications on coffee in the world (DURAN et al., 2017).

In addition to being the world's largest producer of coffee, Brazil is also the one that exports this commodity, which currently represents one of the most important agribusiness products in the country. It is the fifth main product on the Brazilian export agenda and moved, only in 2017, something around five billion dollars. Its production chain employs more than eight million Brazilians, providing income for health and education for workers and their families (BRASIL, 2018).

The consumption of coffee is directly related to its quality, which is commercially determined by the physical characteristics of its beans and their respective sensory properties when transformed into a beverage (PASIN; ALMEIDA; ABREU, 2009). In this sense, sensory characteristics of coffee such as aroma and flavor can be influenced by extrinsic factors, such as temperature and humidity, soil type, growing conditions, and nutritional conditions of the plant, in addition to contamination by deteriorating microorganisms (FRAGA et al., 2003).

Contamination by deteriorating microorganisms is a process that can happen at any stage of the coffee production chain, from harvesting and processing to storage and processing, since in all these stages it is common for the beans to be exposed to sudden changes in the conditions of the environment in which they are (PARIZZI, 2005). Among the main microorganisms found in coffee are bacteria, filamentous fungi (molds), and yeasts (BATISTA, 2001).

It is important here to highlight molds because their association with fruits and coffee beans causes not only economic damage to the production chain but also disorders to human health, as will be discussed below. In general, the presence of fungi can affect the quality of the ready drink due to the deterioration of coffee beans caused by the activity of enzymes, such as cellulases, hemicellulases, xylanases, pectinases, and proteases, among others. This process of deterioration can sometimes generate unpleasant odors and flavors, and still be accompanied by the production of secondary metabolites called mycotoxins (REZENDE et al., 2013).

"Ochratoxin A" (OTA) is the mycotoxin most commonly found in coffee, followed by aflatoxin (BORGES et al., 2009). OTA can be produced by fungi of the genera *Aspergillus* and *Penicillium*, in tropical and temperate regions, respectively (PARIZZI, 2005), especially the species *Aspergillus ochraceus* (*A. ochraceus*) and *Aspergillus carbonarius* (*A. carbonarius*) (CHALFOUN et al., 2007), which are potentially toxigenic.

Many studies have shown that toxigenic fungi are contaminants naturally encoded in coffee (BOKHARI, 2007; NOONIM et al., 2008; SILVA et al., 2000). In addition, the absence of Good Agricultural Practices (GAP) during the cultivation process increases the contamination and development of these organisms, which are dangerous to human health precisely because they can produce mycotoxins (REZENDE et al., 2013), highlighting in this sense, species of the genera *Aspergillus*, *Fusarium* and *Penicillium* (PASIN; ALMEIDA; ABREU, 2009).

Fungi of the genus *Aspergillus* are considered opportunistic pathogens and are often found associated with coffee. In addition to producing OTA, the most toxic of the ochratoxins, they also synthesize aflatoxins, which are capable of causing chromosomal aberrations, unprogrammed DNA synthesis, exchange of chromatids, breaks in chromosomes and bonds with the DNA of human cells (ZUCCHI; MELO, 2009).

Some species of *Aspergillus* are contumacious deteriorators, and therefore capable of causing numerous damages to coffee beans, which can be of a sensory, nutritional, and qualitative nature, such as pigmentation, discoloration, rot and/or development of odors and unpleasant flavors. Among these species, *Aspergillus niger* (*A. niger*) stands out as a widely spread fungus due to its ability to reproduce effectively in different environments, providing strong metabolic diversity and nutritional flexibility (DIOGO et al., 2020).

Mycotoxins, in general, are thermo-resistant metabolites, which means that mycotoxins such as OTA and aflatoxin, produced by *Aspergillus* sp., can remain in coffee beans even after the roasting process (IAMANAKA, 2010). Thus, it seems to be unfeasible to try to eliminate these compounds, although it is possible to reduce their presence to the maximum so that they do not present a risk to human health (BORGES et al., 2009; SOARES; WILD; Venancio, 2013).

The presence of these mycotoxins in coffee can be a consequence of failures in harvesting, processing, unstable drying conditions and inadequate storage of the beans, which accentuates the spread of toxigenic fungi (MELLO, 2016). That said, one of the viable alternatives to reduce or eliminate the possibility of human contamination by mycotoxins in coffee is the improvement of BPA, with suitable storage and transport conditions, as stated by Astoreca et al. (2009), which adds that such actions reduce the growth of toxigenic fungi in coffee used for human consumption.

It should be noted that the presence of known toxigenic fungi in coffee beans does not necessarily imply the presence of their respective toxins, however, it is enough for the nutritional and sensory quality of coffee to be compromised, since, as mentioned earlier, they are decomposing organisms. Thus, isolating and identifying fungi producing these compounds in green coffee beans (before roasting) represents a valuable scientific effort to obtain information on the level of contamination in coffee beans and the danger this poses to the health of people who consume the ready-made beverage.

2 THEORETICAL FRAMEWORK

2.1 FUNGI

Fungi are single-celled (yeast) or multicellular and filamentous (molds or molds), eukaryotic and heterotrophic microorganisms that belong to the kingdom Fungi and that perform important functions in their ecosystems, such as decomposition, symbiosis and pathogenesis. The group of fungi is very diverse after the actual number of species remains unknown. Estimates obtained in the last decade ranged from 2.2 – 3.8 million to 11.7 – 13.2 million species, depending on the methodology used for the calculation. Currently, the number of species described approaches 150,000, a number known to be insignificant compared to the estimates presented by HYDE (2022).

Molds or molds consist of a group of long filaments, called hyphae, which when combined form clusters visible to the naked eye, called mycelia. These can lead to desirable changes in the taste and quality of food, such as in the production of cheeses, for example. In many other cases, however, they cause undesirable transformations, producing unpleasant flavors and odors, which vary according to the degree of deterioration. (SMITH; MALTA, 2016; SOUZA et al., 2017).

Fungi are used in food production, such as fermented and alcoholic beverages, contribute to the pharmaceutical industry, are present in the biodegradation and biological treatment of effluents and are capable of producing enzymes of industrial value and biotransformation. They also have great agricultural and ecological importance, since they maintain the environmental balance, decomposing plant debris, degrading toxic substances and helping plants to grow and protect themselves from enemies, such as other pathogenic microorganisms (ABREU; ROVIDA; PAMPHILE, 2015).

Fungi can spread in the field, during cultivation, harvesting and storage, due to intrinsic factors (substrate) and extrinsic factors (humidity and temperature) (BUENO, 2018). Species of the genus *Aspergillus* are often responsible for causing diseases in plants and animals, and the most common species are *A. niger*, *A. ochraceus* and *A. alliaceus*, being able to contaminate agricultural products at any stage of the production chain (PERRONE et al., 2007).

Despite the numerous positive impacts on the daily life of human life, direct and indirect, fungi can commonly be associated with economic losses, since, as previously stated, they can proliferate in food, causing their deterioration. In addition, some genera aggregate extremely toxic species (*Aspergillus*, *Penicillium*, and *Fusarium*), capable of causing serious damage to humans and animals, through the production of extremely toxic secondary metabolites generically called mycotoxins (ARRUDA; BERETTA, 2019).

2.2 MYCOTOXINS

Mycotoxins are secondary metabolites of low molecular weight, toxic to humans and animals and are produced by several species of filamentous fungi (SOUZA et al, 2017). These substances are expelled through a set of metabolic pathways that are not fundamental to the development and survival of fungi (hence called secondary metabolites) and are biosynthesized when the conditions in which the fungi meet

are suitable for the production of these compounds. Such conditions encompass the presence of substances such as fungicides, favorable environmental changes in physical parameters such as temperature and humidity, as well as biological variables such as susceptibility to the host and the virulence of the pathogen evaluated (BUENO, 2018; MELO, 2014).

The fungi responsible for the synthesis of mycotoxins in agriculture are classified into two groups: field fungi, which affect crops before harvest; and storage fungi, which affect agricultural products after harvest (OLIVEIRA; MATTOS; RODRIGUES FILHO, 2021). The main mycotoxins found in foods are aflatoxins, OTA, patulin, fumonisins, zearalenone, and deoxynivalenol. These are synthesized mainly by species of the genera *Aspergillus*, *Penicillium*, and *Fusarium* (SILVA, 2021). The existence of fungi in food products does not indicate the presence of mycotoxins, however, the absence of visible fungi does not rule out their presence (PRADO, 2014).

Exposure to mycotoxins can happen directly, through the ingestion of plant foods, or indirectly, through foods of animal origin, when they consume the contaminated feed. Some mycotoxins can cause degeneration of the functional capacity of kidneys and liver, while others are neurotoxic or impair protein synthesis, causing a variety of effects, ranging from sensitivity or necrosis of the skin to extreme immunodeficiency (MARROQUIN-CARDONA and t al., 2014).

According to the Food and Agriculture Organization (FAO), it is estimated that about 25% of food and feed worldwide is contaminated with mycotoxins (OLIVEIRA; MATTOS; RODRIGUES FILHO, 2021). When it comes to coffee, OTA is the most relevant and gains special attention, as it has carcinogenic potential (AZEVEDO, 2019).

Due to their physical and chemical properties, mycotoxins are stable chemical compounds, that is, they are thermo-resistant. Therefore, they cannot be eliminated from food through the processing or boiling steps (ROCHA et al., 2020). Specifically, in coffee, they reduce the quality of the drink and can pose health risks. This toxicity led Brazil to establish regulations to ensure the regulation of tolerable levels, and the Collegiate Board Resolution – RDC No. 07, of February 18, 2011, of the National Health Surveillance Agency (ANVISA) provides for the maximum limit of mycotoxins tolerated for food. For roasted coffee (ground or bean) and soluble coffee, the OTA limit is 10 µg/kg (BRASIL, 2011).

2.3 COFFEE QUALITY

In an era of economic and market globalization, it is necessary to increase the competitiveness of the productive sector by investing in the quality of different types of coffee. Currently, the production of high-quality coffee is the best option for Brazilian coffee growing, especially when the focus is on the economic viability of this activity. The quality of a product can be determined through the sum of all the attributes that meet the needs of the consumer. In coffee, these attributes go beyond sensory aspects, with an increasing focus on food security (PRADO, 2014).

Taking into account the substrates that the coffee bean presents (bark, pulp, and seed) it is feasible to grow a diversified microbiota, including filamentous fungi, especially species of *Aspergillus*, *Penicillium*, and *Fusarium*, yeasts, lactic acid bacteria (*Leuconostoc* and *Lactobacillus*) and pectolytic bacteria (*Erwinia*, *Bacillus*) (IAMANAKA, 2010).

Throughout the production cycle, several fungi are associated with fruits and coffee beans and, under favorable conditions, can cause loss of quality, odors and unpleasant flavors, as fungi have a very wide range of enzymatic potential and can also compromise the safety of the final product due to the production of mycotoxins (MELLO, 2016). Several studies conducted in Brazil and abroad have demonstrated the persistent and significant involvement of filamentous fungi as one of the main components of the fruit microbiota after harvest or during drying (AZEVEDO, 2019; GEREMEW, 2016; SANTIAGO, 2020).

In a production system, the harvest and post-harvest processes are fundamental to obtaining a high-quality product. If not managed properly, they can favor the proliferation of fungi that cause undesirable fermentations and can produce mycotoxins, such as OTA, which cause harm to human health (LAZZARINI; MORAES, 1958). No source or type of coffee processing is free from the risk of contamination by toxigenic fungi, including ochratoxigenics. This makes quality issues ubiquitous in their safety and needs to be addressed on a global scale (CHAULFOUN; CORRÊA, 2002).

Coffee is susceptible to infection by ochratoxigenic fungi, especially due to failures during post-harvest harvest. Many coffee growers take advantage of the fruits that fall into the soil after harvest and this procedure, known as sweeping or sweeping, is not recommended from the point of view of health, because it favors the growth of ochratoxigenic fungi existing in the soil, and impairs the quality of the drink (CAMPOS, 2009; SOUZA, 2019).

The harvest presents great importance in obtaining a product of superior quality. This justifies the control of aspects such as the determination of the best time for the beginning of the harvest, the duration period, as well as the appropriate harvest method for the region and/or for the crop tomorrow (EMBRAPA, 2004).

Long periods in which the coffee stays in bags after harvest until it goes to drying, periods of rainfall during the drying stage in yards, lack of turning of the beans in the yard and inadequate storage, are indispensable conditions for the development of fungi potentially producing OTA (BUENO, 2018).

Drying is one of the most important steps in controlling fungi and obtaining a product of desired quality, and if not performed properly, it can lead to defects in the coffee, affecting the appearance and final quality of the beverage (SILVA; ROBERTO; NOGUEIRA, 2007). Coffee is one of the agricultural products that require longer drying time due to the high moisture content of the harvest, thus justifying more efficient drying methods (SIMÕES; FARO-NI; QUEIROZ, 2008).

To prevent fungal growth in grains, it is necessary to dry them thoroughly and keep them in a cool place. When conditions are conducive to fungal development, it is common for it to develop more than one

type of fungus. In the storage period, the grains are constantly colonized by a succession of fungi, according to temperature and humidity. Due to the action of several species of fungi, the grain can be contaminated with different numbers of mycotox-ins (ALMEIDA, 2015).

Batista and Chalfoun (2007) identified a higher level of fungal contamination in sweeping coffee and indicated that dried coffee in an earth yard presents higher contamination by ocratumxin than dry coffee in a concrete yard. Although it is difficult to contain fungi and toxins, it is essential to have the absence of these compounds, since, once produced, the toxin can no longer be eliminated.

Control of the development of toxigenic plants can be achieved through measures aimed at preventing the contamination of crops by fungi in the field and storage. In the field, control is done through BPA's, including the removal of residues from previous crops, crop rotation and use of crop varieties resistant to fungi and other pests. In addition to being fundamental to avoid mechanical damage, such as microcracks and grain breakdowns, during harvest, storage and transportation, it is also necessary to control the damage caused by pests, since all these factors facilitate the development of fungi in grains (SILVA, 2021).

The presence of mycotoxins such as OTA in coffee beans has been a constant concern in importing countries and, therefore, planned hygiene-involving good practices in harvesting, preparation, immediate drying and storage in appropriate environmental conditions and hy-gienically are an important aspect to prevent the introduction and development of OTA producing fungi and ensure the health of the consumer (SILVA; ROBERTO; NOGUEIRA, 2007).

Measures to prevent the occurrence of mycotoxins are recommended through the use of BPA, Good Manufacturing Practices (GMP) and Good Hygiene Practices (BPH). In this way, the risk of contamination and the development of toxigenic fungi can be prevented or minimized, thus reducing the production of mycotoxins. Given the above, the common objective is to identify critical control points to ensure product integrity and sensory quality, and to reduce the risk of ocratoxin contamination of coffee by understanding the stages and conditions of coffee production (CHAULFOUN; CORRÊA, 2002).

3 METHODOLOGY

Five different brands were analyzed, which were purchased in supermarkets located in the municipalities of Inhumas Goiás, Petrolina de Goiás and Santa Rosa de Goiás. These marks were acquired in 1kg packages between March and June 2022, where they were opened in an aseptic environment (laminar flow chapel), moments before the grains were inoculated in a culture medium. From each brand, 40 grains were sampled, which were randomly plated, which were separated into two lots: in one of the lots (50% of the grains) a superficial disinfestation was performed with 1% sodium hypochlorite and 70% alcohol, to eliminate the fungi present on the outside of the grains and evaluate the diversity of fungi that colonize the inside of them; while another batch did not receive the superficial disinfestation with hypochlorite so that

it was possible to evaluate the composition of fungi, which naturally inhabit the outside of the grains or that opportunely infected them on the way between the crop and the processing.

During the disinfestation process, the samples (grains) were first immersed in 1% sodium hypochlorite for two minutes. Then, they were drained and immersed in 70% alcohol for a minute for a more effective disinfestation, being drained again and, as a final step of disinfestation, the coffee samples were washed with distilled and sterilized water to remove residues of sodium hypochlorite and 70% alcohol (OLIVEIRA, 2016).

After disinfestation, all grains were aseptically inoculated in acrylic petri dishes containing BDA culture medium (Potato Dextrose Agar). On each plate was placed only one grain. After inoculation, the plates were incubated in a BOD incubator for seven days at a temperature of 28°C, with a photoperiod of 12 hours. The isolates obtained were purified through repicing, with the aid of sterile tips in an autoclave, transferring them to Petri dishes containing Malt Extract Agar medium and incubated again for seven days, at 28°C of temperature (REZENDE, 2010).

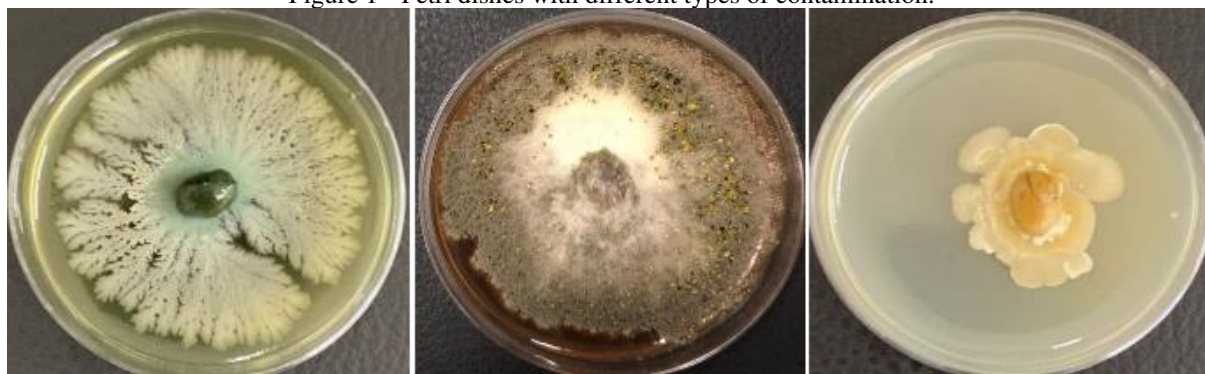
After isolating and obtaining the pure crops, the isolates were identified using the microculture technique, as described in Brasil (2004). After the incubation period, each lamina was carefully removed and placed on a glass lamin containing a drop of cotton blue lactophenol dye, which was taken under a microscope. The identification of each isolate was processed through the observation of morphological patterns traditionally used in these types of studies, as described by Chalfoun and Batista (2003), De Hoog et al (2000), Kern and Blevins (1999) and Pitt and Hocking (2009).

The data obtained were tabulated in electronic spreadsheets through the Excel program.

4 RESULTS AND DISCUSSION

All 200 samples analyzed showed some type of contamination, either by molds or bacteria (Figure 1).

Figure 1 - Petri dishes with different types of contamination.



Source: Own (2022).

In general, high levels of fungal contamination were observed in samples that were not submitted to the disinfestation process, which were contaminated in 99%. On the other hand, of the 100 samples that

underwent disinfestation, only 10% showed some level of contamination. Similar results were observed by Batista et al. (2003) and Rezende et al. (2013), who also evaluated raw coffee beans before and after disinfecting them superficially. This result was expected, since disinfestation removes most of the fungi found on the outside of the grain, favoring only the prevalence of endophytic fungi.

The comparison between the number of contaminated grains of the samples that did not undergo disinfestation (99), and the number of contaminated grains, previously disinfested (11), revealed a reduction of 89.90% of contamination, considering the entire sampling universe used. This result is important because it demonstrates that even by adopting BPA's, and thus reducing most of the contamination of the grains, consumers of coffee is not would be free of toxigenic fungi and their mycotoxins, since endophytic fungi were found in 10% of the grains that went through the disinfestation process.

Taniwaki et al. (2003) analyzed a total of 408 samples of coffee collected and in different stages of processing such as: harvested directly from the coffee tree, harvested from the ground, in the drying steps in the yard, and during storage. The authors found that factors such as the contact of fruits and grains with the ground, poorly performed drying, and inadequate storage, facilitate grain infections by ochratoxigenic fungi. In addition, the respective authors pointed out a low infection rate in coffee fruits harvested directly from coffee trees.

Urbano et al. (2001) studied coffee trees at different stages of maturation and processing, confirming high levels of contamination with OTA-producing fungi such as *A. ochraceus* and *A. niger* in raw grain samples collected in yards and storage. Bueno (2018) emphasizes his study, that the drying of the beans at a safe level improves the quality and health of the coffee, contributing to a longer storage period. The author also states that BPA's are crucial for reducing the fungal incidence of coffee beans.

It is common knowledge among different authors that coffee is very prone to contamination by toxigenic fungi, especially due to failures during the post-harvest process. Among the factors that contribute to the occurrence of contamination can be cited: the delay in drying the coffee beans after harvest, drying in the yards in periods of rain, lack of turning of the beans during drying, in addition to inadequate storage of the beans (BRANDO, 2009; URBANO et al., 2001).

Table 1 presents the numbers of isolates found in the brands analyzed in this work. Among the 100 grains inoculated without disinfestation, 11 presented two different species in each sample.

Table 1 - Numbers of isolates found in raw coffee beans without disinfestation and with disinfestation*.

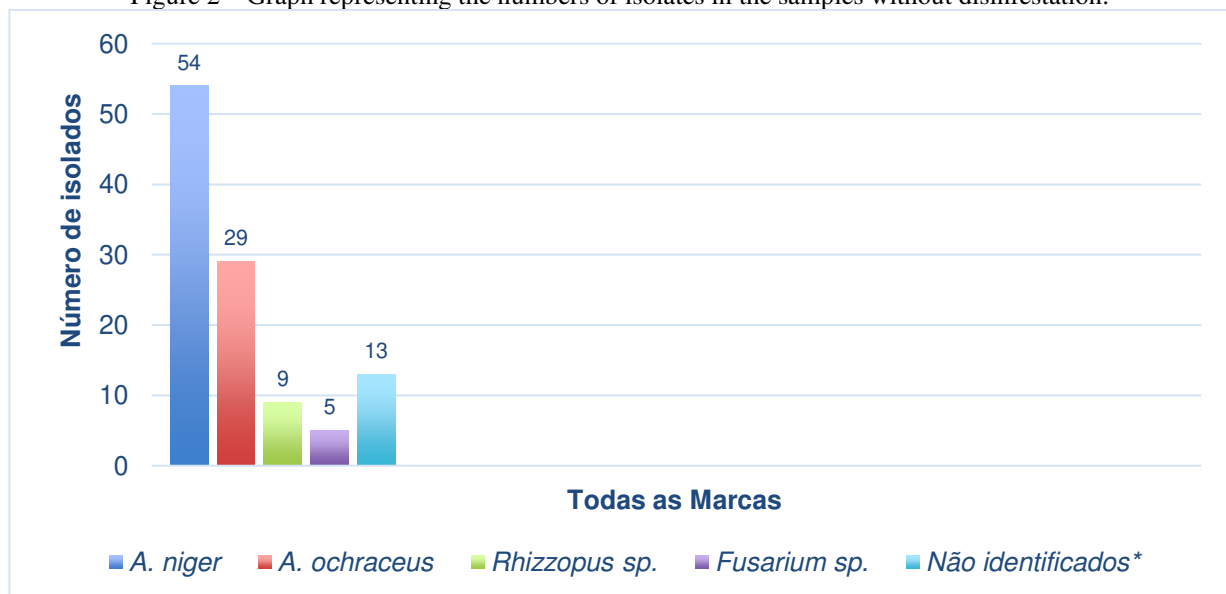
Brands	NO DEWORMING				WITH DEWORM			
	Contaminated plaques	Number of Isolates	Identified species	Unidentified isolates	Contaminated plaques	Number of Isolates	Identified species	Unidentified isolates
1	20	20	<i>A. niger</i> (13) <i>Rhizopus sp. j.</i> (7)	-	08	08	<i>A. niger</i> (5)	03
2	20	23	<i>A. niger</i> (15) <i>A. ochraceus</i> (6)	2	0	0	-	-
3	20	24	<i>A. niger</i> (13) <i>Fusarium sp. nov.</i> (2) <i>Rhizopus sp. j.</i> (1)	08	01	01	<i>Fusarium sp. nov.</i> (1)	-
4	20	24	<i>A. niger</i> (7) <i>A. ochraceus</i> (15)	-	02	02	<i>Fusarium sp. nov.</i> (1)	01

			<i>Fusarium sp. nov.</i> (2)					
5	19	19	<i>A. niger</i> (6) <i>A. ochraceus</i> (8) <i>Fusarium sp. nov.</i> (1) <i>Rhizopus sp. j.</i> (1)	03	0	0	-	0

Source: Own (2022).

Of the five different brands analyzed, 110 isolates were obtained in the samples that did not go through the disinfestation process (Figure 2) and 11 isolates in the samples that were submitted to the disinfestation process (Figure 3).

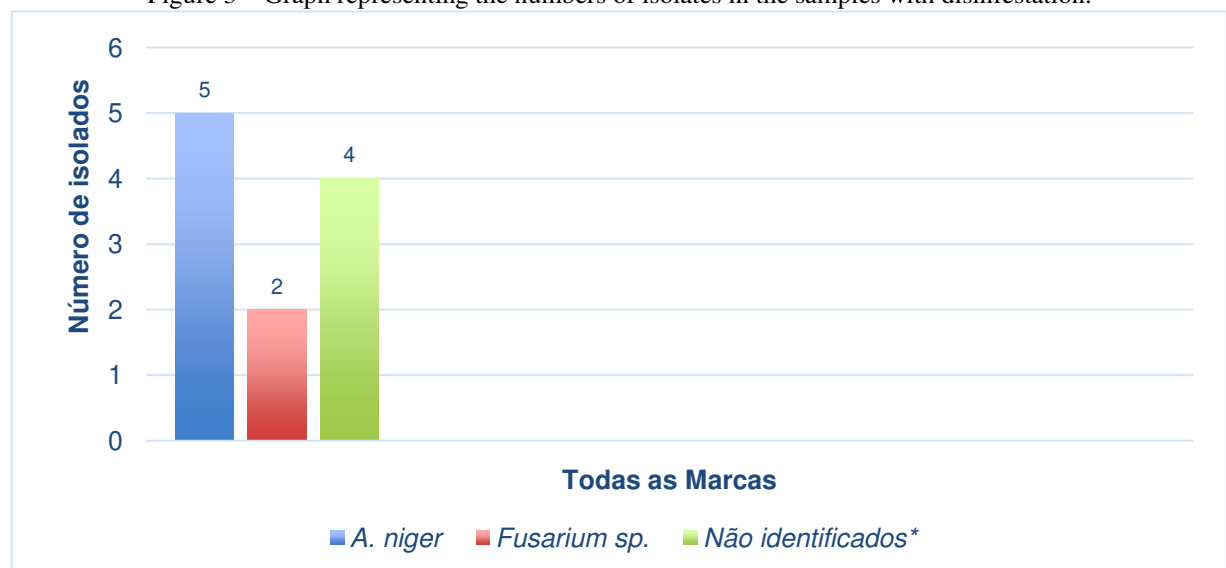
Figure 2 – Graph representing the numbers of isolates in the samples without disinfestation.



* Unidentified isolates presented five different morphologies.

Source: Own (2022).

Figure 3 – Graph representing the numbers of isolates in the samples with disinfestation.



* Unidentified isolates presented three different morphologies.

Source: Own (2022).

Isolates found in the samples that did not go through the disinfestation process showed moderate phenotypic variation, and nine different species were observed, of which five were not identified. Fungi of the genus *Aspergillus* were detected in all the brands analyzed in the present study, with a predominance of *A. niger* (Figure 4a), occurring in approximately 54.55% of the samples, followed by the species *A. ochraceus* (Figure 4b), found in approximately 29.30% of the oysters.

Figure 4 – Isolates of *A. niger* and *A. ochraceus* obtained in raw coffee beans.

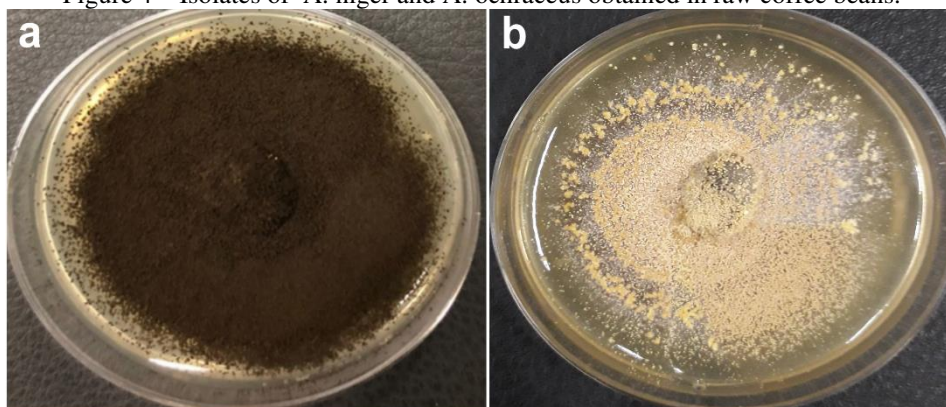


Figure 4 – a – Isolated from *A. niger*; b – Isolated from *A. ochraceus*.
Source: Own (2022).

The occurrence of *A. niger* in coffee beans is not a new fact, having already been described by other authors. For example, Taniwaki et al. (2003) isolated fungi from coffee beans and observed the presence of *A. niger* in 63% of them. Rezende et al. (2013), when studying ochratoxigenic fungi associated with green coffee beans (*Coffea arabica* L.) in conventional and organic cultivation in Brazil, found, in the conventional cultivation system, 31.87% of the beans contaminated by *A. niger*. Oliveira (2016), analyzed coffee samples grown in the South and Cerrado of Minas Gerais, observing the occurrence of *A. niger* in 54% and 71% of the samples respectively.

In addition to *A. niger* and *A. ochraceus*, the grains sampled in this study presented fungi of other important genera such as *Fusarium* (Figures 5a and 5b) and *Rhizopus* (Figure 5c). Previous studies, which also evaluated raw coffee beans, found similar results: Pasin, Almeida, Abreu (2009); Rezende (2010); Geremew et al. (2016); Bueno (2018) and Garrido-Ramírez et al. (2018).

Figure 5 – Isolados of *Fusarium* sp. and *Rhizopus* sp. obtained in raw coffee beans.

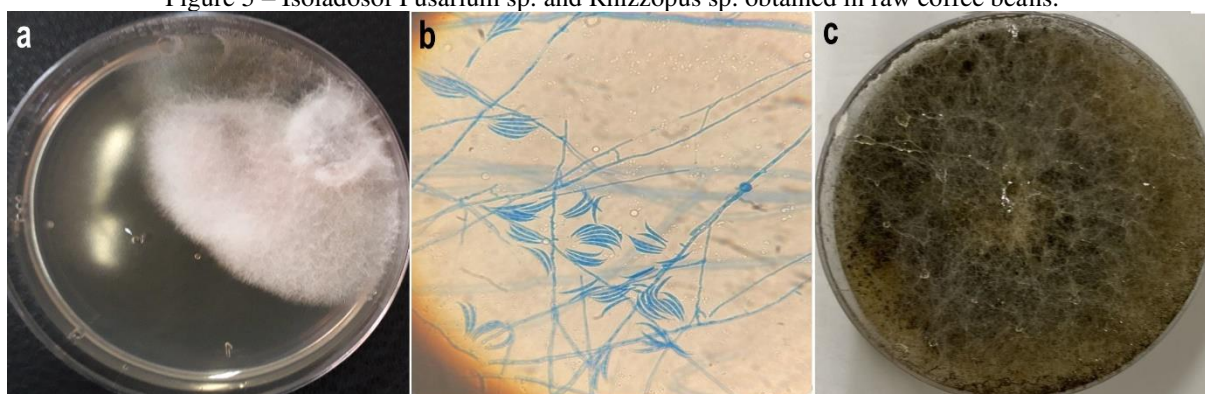


Figure 5 – a – Isolated from *Fusarium* sp.; b – Reproductive structures of *Fusarium* sp.; c – Isolated from *Rhizopus* sp. nov.
Source: Own (2022).

When evaluating raw coffee beans that benefited from two harvests (2016/2017 and 2017/2018) in the state of São Paulo, Cardozo et al. (2019) found that among the identified fungi, isolated from *Fusarium* sp. were detected in both crops. Nakayama et al. (2020), when analyzing the presence of fungi in coffee

beans at different drying times, in the municipality of Patrocínio in Minas Gerais, observed the predominance of *Fusarium* sp. in the pulped cherry coffee, during the entire drying time.

According to Rocha (2020), storage fungi such as *Aspergillus*, *Penicillium* and *Rhizopus* grow rapidly under favorable conditions during cultivation, harvesting, transportation and storage. Christensen and Kaufmann (1965) reported that fungi of the genus *Fusarium* were more common in the bark and mucilage of coffee cherries, while *A. ochraceus* and *A. niger* more often infected stored grains.

Fungi of the genus *Rhizopus* sp. are not producers of mycotoxins, however, they can cause significant losses in the coffee production chain, since their decomposer activity can affect the sanitary, physical, and nutritional quality of the beans (PINTO, 2000). They are highly saprophytic organisms and, in some cases, their incidence can lead to the most destructive of all post-harvest pathogens, and may cause more than 50% of losses during transport logistics (OLIVEIRA; SANTOS FILHO, 2007).

The species *A. niger*, *A. ochraceus* and *Fusarium* sp. are toxigenic fungi, potentially dangerous, capable of producing aflatoxin, OTA and Fumonisin, respectively, and are therefore of high risk to human and animal health (IAMANAKA; OLIVE TREE; TANIWAKI, 2010). Some studies report that *A. niger* are also OTA producer, as in the work of Oliveira (2016), whose results demonstrated that 84% of OTA isolates were identified as *A. niger*.

Rezende (2010) states that in the climatic and environmental conditions of Brazil, among the main OTA-producing species are those belonging to the Nigri Section (*A. niger*) and the Circundati Section (*A. ochraceus*), the latter being the largest producer of OTA. Studies by the authors Palacios-Cabreira et al. (2005), report that raw coffee is an adequate substrate for the growth of *A. ochraceus* and OTA production. Rezende (2010) tested isolates from thirty samples of coffee beans for their ability to produce OTA, resulting in *A. ochraceus* as the main species producing this mycotoxin. Similar results were found by Batista et al. (2003).

According to Soares, Abrunhosa, and Venâncio (2013), aflatoxins have cytotoxic, mutagenic, hepatotoxic, carcinogenic, teratogenic and immunosuppressive effects. OTA, commonly found in coffee, has cytotoxic, nephrotoxic, teratogenic and hepatotoxic effects; while fumonisins have cytotoxic, carcinogenic, teratogenic and hepatotoxic effects (ARRUDA; BERETTA, 2019; MINAMI, 2004).

According to Prado (2017), among the group of Fumonisin, the most toxic and most abundant is Fumonisin B1 (FB1) and is related to leukoencephalomalacia in horses, pulmonary edema in pigs, hepatocarcinoma in rats and has carcinogenic property in humans, causing mainly esophageal cancer.

5 CONCLUSION

The present study presented results that confirmed previous suspicion, corroborating the chosen title. In other words, the results demonstrated that mycotoxin-producing fungi occur in raw coffee beans, marketed in sales and supermarkets in some cities of Goiás. To the brands analyzed, the high incidence of

toxigenic fungi observed points to an unsuitable condition for human consumption, since the possibility of such brands containing mycotoxins in the grains present in their respective packages is high.

It is important to highlight that the coffee brands analyzed may represent problems related to public health, since important species associated with severe disorders in humans, such as *A. niger* and *A. ochraceus* were found, but also, from the economic point of view, since the presence of *Rhizopus* sp. is often associated with sensory losses and the raw material used to make the drink.

It is expected that the results obtained in this research work can serve as a parameter to assist in decision-making by authorities or representatives of the coffee production sector, to achieve improvements in the production process (from the crop to the table), which guarantee the safety and good taste of those who consume this important drink. The judicious adoption of good agricultural practices may be the most viable way for these needs to be met.

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