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Microbiological quality of dehydrated aromatic condiments and spices used in Food: the danger of contamination and level of toxigenic agents

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ABSTRACT

Thirteen types of condiments and aromatic spices (cloves, cinnamon sticks, star anise, fennel, calabrian pepper, ginger, garlic, cumin, black pepper, turmeric, bay leaves, oregano and nutmeg) from street markets and of supermarkets in the city of Cuiabá – MT, Brazil, were evaluated for contamination by fungi and bacteria. Of the 78 plates containing microbiological samples, 68 (87.2%) presented a positive result, isolating 58 species, 43 (74.1%) of filamentous fungal species, 10 (17.2%) of yeast-like fungal species, with two groups identified only at the genus level and 5 (8.6%) bacterial species identified. The values of colony-forming units per gram of product (CFU/g) detected in samples of condiments sold in free fairs and industrialized markets were higher than those of the federal reference standard, varying up to 8.2×10^4 CFU/g. There was no significant difference between these values. *Aspergillus* spp. was the most isolated genus, with the species *Aspergillus niger* predominating, followed by species from the genus *Penicillium, Cladosporium* and *Fusarium*. Among the yeasts found, the most prevalent genera were *Candida* spp., *Saccharomyces* spp. and *Rodothorula* spp. with the highest amount of CFU/g isolated in cinnamon bark samples. The bacteria were the least isolated, with the highest CFU/g value coming from the cumin *Streptococcus gengivalis* ($3.2\text{x}10^3$). The condiments analyzed in this study showed contamination by potentially pathogenic fungi and mycotoxin producers, the isolated bacteria may pose a risk to the health of consumers.

Introduction

According to the time that we evaluate the intertwined tapestry of interactions that exists in the world, every day it becomes more evident that microorganisms are responsible for organizing life on the planet. It is irrefutable that fungal and bacterial microorganisms play a fundamental role in any environment where they are present. Condiments and spices are an integral part of the daily diet, used for medicinal purposes, in the practices of religious rites, as cosmetic and perfumery uses.

Since ancient times, spices have been used in various cuisines around the world (Brazilian, Egyptian, Chinese and Indian mainly) due to their coloring and flavoring properties, without discarding their antimicrobial, antioxidant, preservative, medicinal, cosmetic and nutraceutical properties (Iha and Trucksess, 2019). Even though they have constituent chemical compounds such as alkaloids, coumarins, flavonoids, glycosides, isoflavonoids, tannins and terpenes, contamination by microorganisms is inevitable (Gurtler and Keller, 2019).

This may be due to the place of cultivation, harvesting and processing, considering that most spices are grown in tropical countries with high temperatures and humidity, ideal for the growth and spread of fungi and the production of mycotoxins.

Therefore, at the time of pre-harvest, pest and insect infestation in spices contribute to fungal contamination and once they are infected in the field, the fungi will continue to grow during storage when under favorable conditions. Factors linked to processing such as drying in the sun, considered profitable, can result in the growth of fungi, leading to deterioration and loss of quality and safety of spices (Syamilah *et al.,* 2022).

According to the Food and Drug Administration (FDA), spices are defined as "any aromatic vegetable that is whole, crushed or ground, except those substances traditionally considered foods whose use in food is as seasoning rather than nutritional" (Center for Food Safety and Applied Nutrition, & Office of Regulatory Affairs, 2023).

In Brazil, RDC nº 276 of September 22, 2005, provides for the Technical Regulation for spices, seasonings and sauces, complemented by the FDA, which defines those spices "are products made up of parts (roots, rhizomes, bulbs, peels, leaves, flowers, fruits, seeds, stalks) of one or more plant species, traditionally used to add flavor or aroma to foods and drinks" (Brasil, 2005).

In this process, even though the water activity (Aw.) of the food is low, xerophilic fungal species of *Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium* and *Rhizopus* are easily found (Syamilah *et al.,* 2022; Kortei *et al.,* 2022a; Nurtjahja *et al.,* 2019; Pickova *et al.,* 2020). Bacterial endospores of Bacillus and Clostridium, (Mathot *et al.,* 2021; Yehia *et al.,* 2022) Crononbacter (Liu *et al.,* 2018), and yeasts such as Rhodotorula (Kortei *et al.,* 2022b) are also amenable to growth and isolation.

The normative instruction in force in Brazil, in No. 161, of July 1, 2022, from the National Health Surveillance Agency ANVISA/MS, defines microbiological standards for all food groups. For spices, ANVISA defines a maximum standard of 5×10^2 CFU/g of *Escherichia coli* and the absence of Salmonella in 25g (Brasil, 2022).

A worrying fact is the absence of established limits for filamentous fungi and yeasts in spices in Brazilian legislation. Considering that many condiments are consumed fresh, contamination values above those established by the Agência Nacional de Vigilância Sanitária (ANVISA) can contaminate other foods at the time of preparation and, as a result, can cause damage to the consumer's health.

In view of the above, it is understood that knowledge of fungal contamination in food products is extremely important as a way of certifying their safety and quality. Thus, the objective of the present study was to microbiologically analyze thirteen dehydrated condiments sold in free fairs/free markets (open air markets) and supermarkets (retail markets) in the city of Cuiabá-MT, Central region of Brazil.

Materials and Methods

Samples

A total of 78 samples of the following dried spices were analyzed: dried clove flowers (*Sygygium aromaticum* L.), cinnamon sticks/barks (*Cinnamomum verum* J. Prezi = *C. zeylanicum* Ness), dried star anise flowers (*Illicium verum.* L.), flakes of pepperoni pepper or calabrian pepper (*Capsicum baccatum* L.), slivers of dehydrated ginger (*Zingiber officinale* Rosc.), slivers of dehydrated garlic (*Allium sativum* L.), cumin seeds (*Cuminum cyminum* L.), fennel seeds (*Pimpinella anisum* L.), black pepper seeds (*Piper nigrum* L.), turmeric powder (*Curcuma longa* L.), dehydrated bay leaves (*Laurus nobilis* L.), dehydrated oregano leaves (*Origanum vulgare* L.) and nutmeg shavings (*Myristica fragrans* Houtt.), which are used as condiments and spices in Brazilian cuisine and mainly in typical Mato Grosso dishes, in the region Midwest of Brazil (Table 1).

These 78 samples of condiments and spices were obtained from three free fairs and three supermarkets in the city of Cuiabá, state of Mato Grosso, Central region of Brazil. At each fair, 13 samples of dried spices and condiments were purchased, totaling 39 samples for analysis. The same was done for the three supermarkets chosen in different parts of the city of Cuiabá - MT, which were chosen according to the amount of population flow in the location and the offer of the products evaluated (Table 1).

The samples were packed in their original packaging, identified and transported in a Styrofoam box (EPS) sanitized with 70% alcohol, with ambient temperature (23º-25ºC) and humidity (2%-3%) inside the box, evaluated using a digital thermo-hygrometer (INCOTERM).

Sample Preparation

Sample Preparation the samples were taken to the Microbiology Laboratory at Faculdade Educare, where fungal identifications were carried out and part of the analyzes were carried out at the Carlos Chagas Laboratory, of the Sabin Group, for bacterial identifications; both institutions are located in the city of Cuiabá-MT. To analyze the isolated microorganisms, the modified methodology of Silva *et al.,* (2017) and Pitt & Hocking (1999).

Approximately 200g/sample of each condiment and/or spice was purchased and packaged in sterilized polyethylene bags and stored in a refrigerator at -4ºC until further use. They were aseptically evaluated and subjected to specific preparation.

Samples of cloves, star anise, fennel, cumin, oregano and black pepper were macerated in a porcelain crucible with the help of a pestle (mortar), in order to reduce the samples into smaller particles. The bay leaves, cinnamon, nutmeg, pepperoni pepper/calabrian pepper, ginger, garlic, which were slivers, peels, leaves or flakes were crushed in a blender, until they were smaller to facilitate manipulation during handling. The powdered condiments, turmeric, were processed according to how they were acquired. The blender glass jar/cup was disassembled after each use for cleaning. A porcelain crucible and its respective pestle were used for each of the analyzed samples and after each use, all objects and their parts were washed with soap and water, rinsed in common water and finally rinsed in sterilized distilled water and sanitized with 70% alcohol and sterilized at 125ºC, leaving to dry in an oven at 35ºC, thus ensuring aseptic analysis.

Sample analysis

According to the modified methodology of Silva (2017), 25g of each sample was weighed, adding a volume equal to 225 mL of 0.1% peptone saline solution for subsequent dilutions (10^{-1}, 10^{-2} and 10^{-3}).

For sowing, the "Pour Plate" spread plate technique was used with the aid of a Drigalski strap, inoculating 0.1 ml of each dilution on the surface of Petri dishes containing BD Difco™ Sabouraud dextrose agar plus chloramphenicol (100 mg/L) (INLAB). The plates were incubated at room temperature (25 ± 2 C) for 3 to 10 days for the growth and counting of filamentous and yeast-like fungi, observed every day after plate inoculation. All analyzes were performed in triplicate and total colonies were counted, and the results were expressed in colony forming units (CFU/G).

For the purpose of bacterial isolation, 0.1 mL was inoculated onto the surface of Petri dishes containing properly sterilized and identified blood agar, chocolate agar and MacConkey agar (DIFCO™). After spreading over the surface of the culture medium, the plates were incubated inverted in a bacteriological incubator at 36±1 C/48 hours. Counting was carried out every 24/48 hours, calculating the average number of colony forming units (CFU/g).

Morphological assessment ad identification

After the growth of the fungal colonies, they were counted, and the identification of the structures was carried out according to standardized methods for identifying filamentous fungi using the microculture technique (Riddell, 1950). The fungi were evaluated according to their microscopic and macroscopic characteristics (diameter, texture, pigmentation, surface and reverse appearance, shape and size of structures and conidia) by Lacaz *et al.,* (2019). For bacterial identification, after colony isolation, Gram staining and necessary biochemical tests were performed according to Koneman (2018).

Results and Discussion

Spices and condiments such as black pepper, fennel, cinnamon, cloves and other seasonings are widely used as flavorings and flavor enhancers in Brazilian cuisine. Spices are exposed to microbial contamination during pre-harvest, post-harvest and storage period and throughout the handling and transportation process. Tropical countries such as Brazil suffer losses mainly due to fungi during storage.

A total of 65 species and 24 genera of fungi and bacteria were isolated from the tested spices. Among the fungi, the following were isolated: *Aspergillus terreus, Eurotium amstelodami, Penicillium glabrum, Syncephalastrum racemosum, Aspergillus niger, Fusarium oxysporum, Paecilomyces variotti, Cladophialophora carrionii, Curvularia alternata*, among others fungal specimens (Figure 1), were commonly isolated from all 78 samples of condiments and spices collected in free fairs and supermarkets in the city of Cuiabá – Mato Grosso – Brazil (Table 2 and 3).

The genus Aspergillus constitutes one of the dominant storage fungi represented, in this study, by 14 species: *Aspergillus niger, A. ochraceus, As. flavus, A. terreus, A. nidulans, A. fumigatus, A. flavus, A. tamarii, A. parasiticus, A. oryzae, A. sydwii, A. ustus,* together with species of the genus *Eurotium, E. amstelodami, E. herboriorium and E. repens* (Figure 1). The fungi isolated in the test samples belong to the Ascomycetes phylum (76.9%), showing predominance among the isolated species; followed by the Basidiomycetes phylum (9.2%) and finally the Mucoromycota (=Zygomycota) (6.2%), followed by isolated bacteria belonging to the *Proteobacteria phylum*, Enterobacteriaceae family (3.1%) and the *Firmicutes phylum* (4.6%) representing the Streptococcaceae family and Staphylococcaceae family of the isolates.

The average count of colony-forming units per gram (CFU/g) in spice samples collected at different fairs and local supermarkets in Cuiabá-MT were presented (Tables 2 and 3), respectively. Among the samples from fairs with the highest average count, found in carnation flowers found in Fair 1 contaminated with *Aspergillus nidulans* (7.5 x 10^4 CFU/g) and supermarket samples, there were tumeric flakes, with *Aspergillus niger* (8.2 x 10^4 CFU/G). The lowest average count was found in black pepper samples identified at Fair 3, contaminated with *Cladosporium cladosporioides* (1 x 10^2 CFU/G) and supermarket samples, were found in star anise samples, contaminated with *Eurotium amstelodami* (1.2 x 10^2 cfu/g) (Table 2 and 3).

Yeasts and bacteria were also detected. Yeasts were present in samples of Cinnamon peels at Fair 1, isolating *Candida krusei* (2.8 x 10^3); *C. parapsilosis* complex (1.1 x 10^3), star anise in fair 2, isolating *C. guilliermondii* (1.52 x 10^3), in bay leaves in fair 3, *C. albicans* (2.7 x 10^3), in saffron, isolated in fair 3, identifying *Sacharomyces cerevisiae* (1.66 x 10^2), chips in ginger, isolated in fair 1, *Sacharomyces cerevisiae* (1.2 x 10^3).

In the supermarket samples, yeasts were isolated in samples of cinnamon (supermarket 1, *Trichosporon* spp. 1.5 x 10^3), star anise (supermarket 2, *C. albicans* 2.4 x 10^3), cumin (supermarket 2, *C. tropicalis* 8.5 x 10^2), nutmeg (supermarket 1, *C. krusei* 1.5 x 10^3; *Rhodotorula glutinis* 3.5 x 10^3), turmeric (supermarket 2, *R. mucilaginosa* 1.2 x 10^3), ginger (supermarket 2, *R. mucilaginosa* 2.2 x 10^3), garlic (Supermarket 1, *S. cerevisiae* 2.1 x 10^3; supermarket 2, *R. mucilaginosa* 7.2 x 10^2) and pepperoni pepper (supermarket 2, *R. glutinis* 2.5 x 10^3) (Table 2 and 3).

Many bacteria were isolated in samples from fairs: star anise (fair 1, *Escherichia coli* 2.7 x 10^2; fair 2, *Streptococcus mutans* 1.4 x 10^3), cumin (fair 2, *S. gingivalis* 3.2 x 10^3; *E. coli* 1.5 x 10^3), bay leaves (fair 2, *Salmonella gallinarum* 1.93 x 10^3) and turmeric (Fair 1, *S. aureus* 1.9 x 10^3; *S. mutans* 2.5 x 10^3). In the supermarket samples, bacteria were detected from garlic (supermarket 1, *Staphylococcus aureus* 2.4 x 10^3) and pepperoni pepper (supermarket 2, *S. aureus* 2.1 x 10^3) (Table 2 and 3).

A curious fact observed in the analyzes was the prevalence of bacteria in samples obtained from supermarkets, such as Gram-positive cocci (*S. aureus, S. mutans, S. gengivalis*) and Gram-negative bacilli (*E. coli, S. gallinarum*). In supermarket samples, yeasts showed greater isolation, with the species *C. krusei, C. parapsilosis, C. tropicalis, Cryptococcus* spp., *R. mucilaginosa, R. glutinis, S. cerevisiae* and *Trichosporon* spp. being isolated, with greater isolations for *R. mucilaginosa*, among the group of yeast-like fungi.

When comparing the fungal species that were present in samples from street markets and supermarkets for the same condiment/spice, we can observe that the one with the highest number of contamination was the spice cinnnamon sticks (*Aspergillus niger, A. nidulans, A. flavus, A. terreus, A. fumigatus, Penicillium citrinum, Fusarium oxysporum, F. verticillioides, Eurotium herbariorum, Candida krusei, Rhodotorula mucilaginosa, Cladosporium herbariorum, Curvularia clavata* and others), followed by garlic (*A. niger, A. ochraceus, A flavus, A. terreus, A. tamarii, Cladosporium cladosporioides, Sacharomyces cerevisiae, Staphylococcus aureus, R. mucilaginosa* and *Purpureocillium lilacinum* and others) and peppers (*A. nidulans, A. niger, A. ocraceus, P. citrinum, C. cladosporioides, Alternaria alternata, Staphylococcus aureus, Paecilomyces viridis, Penicillium crustosum, F. oxysporum* and other species) (Table 1). The condiments/spices with the least contamination were black pepper, finding exclusively *Penicillium sydowii* and *P. italicum* in this spice, as shown in Table 1.

The table was constructed with the most prevalent microorganisms, it is noteworthy that bacteria and yeasts were not mentioned due to their low prevalence. Among the bacteria, *E. coli, S. gallinarum, S. aureus, S. gingivalis* and *S. mutans* were isolated, and among the yeast fungi the genera *Rhodotorula, Sacharomyces, Trichosporon, Cryptococcus a*nd the most prevalent yeasts of the genus Candida, with emphasis on *C. krusei.*

Regarding the fungal species isolated from 78 samples collected, *Aspergillus niger* (23.1%) was the most isolated fungal species, as shown in Figure 2. Other species of the genus Aspergillus, such as *A. ochraceus* (17.6%), *A. flavus* (16.5%), *A. terreus* (13.2%) and *A. nidulans* (9.9%), were isolated in lower percentages and categorized among the ten most isolated, followed by other Aspergilli such as *A. fumigatus, A. tamarii, Eurotium amstelodami*, *E. herboriorum* and *A. parasiticus* (Figure 2).

Isolation of Filamentous Fungi

Twenty fungal genera composed of 60 species were obtained from 78 samples of spices and condiments acquired from free fairs and supermarkets in the city of Cuiabá/MT, Central region of Brazil, in January 2020.

We know that condiments are obtained in different ways and are commonly exposed in environments, which leads to contamination, generating microbial multiplication. These spices, also associated with storage conditions, favored by contamination by insects, rodents and birds, by the sweat of hands upon contact, odors induce organic sensitivity causing sneezes that project saliva particles (Flügge droplets) and even fragments of dust, soot, airborne fragments; associated with favorable environmental conditions, humidity and temperature, favor the development of fungi and bacteria that benefit from this adequate condition, promoting their proliferation (Iha and Trucksess, 2019).

Pitt & Hocking (1999) emphasize that fungi of the genera Aspergillus, Penicillium and Eurotium are often the dominant microbiota of dried spices, whether whole or crushed. More currently, the filamentous fungi of the genera *Aspergillus, Penicillium, Fusarium* and *Cladosporium* are those that are most frequently present in spices, and some species belonging to these genera are known for their ability to produce different mycotoxins such as aflatoxins (AFs), zearalenone (ZEN), ochratoxins (OTA), trichothecenes (TCTs) and fumonisins (FBs) and patulins (PAT) that have toxic, carcinogenic, mutagenic and teratogenic effects in humans and animals (Hashem and Alamri, 2010).

Research carried out by Romanian researchers showed the power of aflatoxins in commercialized spices, finding aflatoxin B1 present in all tested products, mainly in black pepper (average value 126.3 ng/g); ochratoxin A, present in sweet pepper (average value of 328 ng/g) and zearalenone in pepperoni pepper (average value of 604 ng/g) and sweet pepper (average value of 382 ng/g) (Man *et al.,* 2016).

In Brazil, in 2018, (Garcia *et al.,* 2018) found in their results, a high frequency of potentially toxigenic species in white and black peppers with the presence of aflatoxigenic and ochratoxigenic fungi.

Rosemary and fennel showed contamination with aflatoxin B1 ($\rho<0.01$) and isolation of *A. flavus*. Ochratoxin A was not detected in the samples, even with the presence of fungi.

Italian researchers, evaluating spices and culinary herbs in cities in Italy and Tunisia, the results showed the existence of mycotoxins in samples of coriander, bay leaves, mint, rosemary and verbena. In both geographic origins, different contamination was detected. Among the mycotoxins investigated, Aflatoxin (AFB2), Aflatoxin

(AFG1, AFG2), Trichothecenes (T2 and HT-2), while none of the samples contained AFB1 and Fumonisin (FB1) (Potortì *et al.,* 2020).

According to the European Union standard, the maximum limit of AFB1 in spices such as black pepper, paprika, nutmeg, ginger, turmeric and a mixture of spices is 5 µg/kg, while the sum of AFB_1, AFB_2, AFG_1 and AFG_2 in spices is 10 µg/kg (European Commission, 2010). However, the maximum regulated limit of OTA in spices is 15 µg/kg (European Commission, 2012).

In 2021, the European Commission published the results of the first coordinated control plan on the authenticity of aromatic herbs and spices launched by the Directorate-General for Health and Food Safety (DG SANTE) and implemented by 21 EU Member States, Switzerland and Norway. The percentage of samples considered at risk was 17% for black pepper, 14% for cumin, 11% for turmeric and saffron and 6% for paprika. Oregano was identified as the most vulnerable, with 48% of samples at risk of contamination. (Maquet *et al.,* 2021)

In Brazil, RDC no. 7 of February 18, 2011, provides for the maximum tolerated limits (LMT) for mycotoxins: aflatoxins BG (1 to 20µg/kg), aflatoxin M1 (0.5 to 5µg/kg), ochratoxins (2 to 30µg/kg), deoxynivalenol (200 to 3000µg/kg), fumonisins (200 to 2000µg/kg), zearalenone (20 to 1000µg/kg) and patulin (50µg/kg), in foods for human consumption (Brasil, 2011).

We can observe that the surprising power of these fungi in food is devastating. Historical reports from the 1960s, more than 100,000 turkeys died in England due to hepatotoxic and hepatocarcinogenic poisoning accompanied by internal bleeding and liver necrosis, due to ingestion of flour peanuts of Brazilian origin (Goldblatt, 1969).

The majority of the producing species belonged to the genus Aspergillus and the section Flavi, particularly *Aspergillus parasiticus, A. flavus* and *A. nomius* (Rodrigues *et al.,* 2009). Nowadays, Arab researchers confirmed the isolation of these three species of the genus Aspergillus in their series in 2014 in Qatar (Hammami *et al.,* 2014), isolated from spices in supermarkets.

Specimens of fungal species have been causing a devastating apocalypse in agriculture, causing a multitude of pathogenic manifestations in animals and plants such as rice blast (*Magnaporthe grisea*), wheat leaf spot caused by *Bipolaris sorokiniana, Drechslera tritici-repentis, Stagonospora nodorum* and *Septoria tritic*i and corn rust (*Puccinia polysora* and *P. sorghi*), affecting the entire global ecosystem.

However, other fungal microorganisms have specialized in affecting a variety of animal species, causing extinction and threats to amphibians (*Batrachochytrium dendrobatidis*), turtles (*Fusarium solani*), bats (*Pseudogymnoascus destructans*) and even corals (*Aspergillus sydowii*), others until recently time were unknown to the world, as is the case of the yeast *Candida auris*, which existed free in the environment and became a human pathogen favored by global warming, the dimorphic fungus, *Sporothrix brasiliensis*, a devastating entity that can affect the organs and systems of beings humans and animals and *Aspergillus flavus*, which has become resistant due to the indiscriminate use of agricultural pesticides, promoting the susceptibility of the effectiveness of antifungals used in human clinics.

According to the Food and Agriculture Organization of the United Nations (FAO, 2023), approximately 25% of food produced in the world is contaminated with mycotoxins, generating annual losses of one billion dollars.

In developed countries, the legislation in force is very restrictive and the most common health problems associated with mycotoxins are related to the appearance of tumors and the weakening of individuals' immune systems, which reduces their resistance to infectious diseases (FAO, 2001; Gil *et al.,* 2016).

In Brazil and in countries still considered underdeveloped, exposure to mycotoxins occurs more easily since agricultural practices, storage methods and legislation are inadequate (Wild and Gong, 2010), leading to exposure to the population living in these countries to mycotoxins.

Several studies indicate that black pepper is the condiment with the highest number of microorganisms isolated. Second, Freire *et al.,* (2000) this condiment is mentioned because it contains a high microbial load, although all spices are subject to microbial contamination.

Evaluating this condiment, in a study carried out by Gatti *et al.,* (2003) isolated a large number of fungal species from 115 samples of black pepper, the xerophilic species *Eurotium chevalieri, E. rubrum* and *E. amstelodami,* were the most evident fungi, followed by *Aspergillus flavus* and *A. niger* and also *A. tamarii, A. carbonarius, A. fumigatus, A. sydowii, A. restrictus*, *A. ochraceus* and *A. parasiticus* were isolated. The genera *Penicillium, Cladosporium, Curvularia, Rhizopus, Emericella* and *Paecilomyces* were also present.

Researchers in Turkey found filamentous fungi (*A. flavus, A. fumigatus* and *A. niger*) and yeast-like fungi of the genus Candida, *C. albicans* and *C. tropicalis* in varied samples of peppers (Vural *et al.,* 2004). The isolation records of these fungal entities are in accordance with those isolated in this study.

In our study, the presence of 20 filamentous and yeast-like fungal genera identified at the species level was identified in the spices and condiments evaluated. Turmeric from supermarkets had the highest fungal contamination (8.2 x 10^4 CFU/g), while black pepper from free fairs, star anise and ginger, obtained from supermarkets, had the lowest counts. (1.2 x 10^2UFC/g).

Hammami *et al.,* (2014) in the Arabian Peninsula (Continental Asia), reported that among the spice samples tested in their sample, pepper powder showed a greater presence of fungal propagules, while ginger, curry and garlic samples did not show satisfactory results. The results obtained by Freire *et al.,* (2000) reported 42 species of fungi on black and white peppers.

Aspergillus flavus was the predominant species, followed by *A. niger, Chaetomium globosum, Penicillium brevicompactum, E. nidulans, Eurotium* spp. and *P. glabrum*.

(Syamilah *et al.,* 2022), in a review of fungal mycotoxins in spices, found *A. flavus* and *A. niger* to be the most commonly isolated fungi in 13 species of foods used in Asian cuisines. The results of this study are consistent with the quantity of spices, fungal genera and diversity of fungal entities isolated in this study.

In 2014, Teixeira-Loyola *et al.,* (2014) in Minas Gerais, Southeastern Brazil, when they analyzed fungal contamination in spices such as basil and black pepper, they found a high level of contamination in black pepper, while oregano presented a lower value compared to them.

In the Central-West region of Brazil, samples of spices such as black pepper, cinnamon, oregano, fennel and coloriferous, also called urucum/annatto (*Bixa orellana* L.), sold in free and permanent markets in Cuiabá/MT, were analyzed and found, the highest frequency of microorganisms was found in black pepper, followed by coloriferous, cinnamon, fennel and oregano. The prevalent genera were *Aspergillus, Penicillium* and *Paecilomyces*. Species, such as *A. flavus, A. ochraceus, A. oryzae* and *A. parasiticus*, are often cited as producing mycotoxins (Cunha Neto *et al.,* 2013).

In the same region, researchers from Mato Grosso, in 2016 (Oliveira *et al.,* 2016), analyzing condiments from the Porto market, a very popular local market in the central region of Brazil, found the highest count in basil and black pepper of fungal contamination (>100 CFU/g) and, in rosemary, the lowest count (2.8 x 10^1 CFU/g).

In 2017, other researchers from Mato Grosso isolated six fungal genera and three of them considered to be of clinical importance *Aspergillus, Penicillium* and *Fusarium*. These researchers concluded that the spices evaluated were within the values established by ANVISA, however the fungal analysis showed a large amount of CFU/g, including species reported in the literature as toxigenic (Ormond de Oliveira *et al.,* 2017).

Other Brazilian researchers, in samples of spices sold at fairs in the city of Campina Grande/Brazil, recorded 78% of fecal coliforms, and in 22% the numbers were higher than 1,100 MPN/g. Black pepper was one of the products that showed the highest count of microorganisms, being outside the established microbiological standards (Silva *et al.,* 2013).

A study carried out in Teresina in Piaui, northeast region of Brazil (Silva *et al.,* 2015), black pepper and annatto (*Bixa ollerana* L.), collected from free fairs and supermarkets in the city of that region, showed high positivity rate for filamentous fungi such as the genus Aspergillus spp. (9 species), followed by the genus Penicillium (3 species).

Zi (2014) found the highest number of isolated fungi recorded in Coriander, and the lowest observed in Cumin. Evaluating samples of cardamom, cumin, black pepper, coriander, cinnamon, ginger, anise, turmeric, fennel, nutmeg, among others, this researcher reported the presence of fungi, with the genera *Aspergillus, Penicillium, Altern*aria and *Fusarium* prevailing.

That same year, researchers from the Brazilian Amazon collected samples of rosemary, oregano, annatto (coloriferous), garlic, black pepper, coriander, cumin, saffron, sage, basil, marjoram and chives, finding marjoram to be of greater value against molds and yeasts among the samples researched (Walker *et al.,* 2014). In the city of Limoeiro do Norte, in Ceará,, Northeast region of Brazil (Uchoa *et al.,* 2019), found values that differ from those found in this study. All samples analyzed showed contamination, but with values below the standard required by legislation (5.0 x 10^2 $NMPg^{-1}$).

Different terms are used to refer to capsicum fruits known as “red pepper”, “pepper”, “spicy red pepper”, “Tabasco pepper”, “paprika pepper” and “cayenne pepper”. Brazilian and Italian researchers reported an infinite list of fungal species derived from pepper products (*Capsicum* spp.), in addition to the genus Aspergillus, other genera such as: *Alternaria, Cladosporium, Epicoccum, Fonnellia, Fusarium, Penicillium, Rhizopus, Trichoderma, Mucor, Mycosphaerella, Scopulariopsis, Syncephalastrum, Ulocladium, Wallemia* and *Eurotium* and their mycotoxins have been reported (Costa *et al.,* 2019). Man *et al.,* (2016), in Romania, observed fungi in 72.7% of black pepper samples; 33.3% in white pepper; 30% on sweet pepper and 25% on hot pepper products. The most common fungus isolated was from the genus Aspergillus.

Czech researchers (Klimesová *et al.,* 2015) evaluating spices in that country, the fungal genera *Aspergillus, Penicillium, Mucor, Phoma* and *Alternaria*, and the species *Aspergillus flavus*, as the most isolated contaminating agent in the samples allspice, pepper black pepper, cumin, bay leaves and coriander, as well as researchers from Indonesia (Nurtjahja *et al.,* 2019) found the highest number of *Aspergillus flavus* isolates found in white pepper, followed by nutmeg, cardamom and black-pepper.

In 2020, Iraqi researchers (Al-abas *et al.,* 2020) found high isolation of *Aspergillus flavus* (41.7%) followed by *Aspergillus niger* (25.9%) and *Aspergillus parasiticus* (12.9%). More recently, in Romania (Cighir *et al.,* 2023), researchers analyzed twenty-one samples of seasonings and spices and found 28.57% of the tested samples presented different degrees of fungal contamination, mainly with Aspergillus section Flavi, followed by Aspergillus section Nigri and other fungi in smaller quantities. The information found by these researchers is in line with the results found in this study were species of the genus Aspergillus, such as *A. niger, A. flavus, A. terreus, A. ochraceus* and *A. nidulans* (Figure 2) graph are also proved to be efficient agents in contamination of spices and condiments.

The levels determined in the fungal count around Cinnamon sold in supermarkets and free fairs in the State of Paraná/Brazil (Furlaneto and Mendes, 2004), found the presence of fungi in dehydrated spices sold in free fairs. These authors recorded equal values for cinnamon peels (2.7 x 10^3 UFC/g), oregano (3 x 10^4 UFC/g) and basil (1.7 x 10^5 UFC/g). In our sample, cinnamon sticks were also shown to be the spice most prone to contaminating propagules, finding the highest values in free fairs for *Cladosporium cladosporioides* (5.2 x 10^2) and in supermarkets for *Aspergillus flavus* (6.5 x 10^3) (Table 2 and 3).

Hashem and Alamri (2010), evaluating spices sold in Saudi Arabia, detected *Aspergillus niger, Purpureocillium lilacinus, Rhizopus stolonifer* and *Ulocladium chartarum* in cinnamon samples. Teixeira-Loyola *et al.,* (2014) evaluated samples of cinnamon powder collected from fairs and supermarkets and determined counts between 1 x 10^5 and 6 x 10^2 CFU/g of contamination and found the genera *Aspergillus, Penicillium* and *Cladosporium*.

In our series, samples of Cinnamon sticks were presented in street markets, finding the species *Eurotium repens, Curvularia clavata, Candida krusei (=Pichia kudriavezevii/Issatchenkia orientalis), Aspergillus flavus, Penicillium citrinum, A. fumigatus, A. terreus, C. cladosporioides, C. parapsilosis* and in supermarket samples, *Trichosporon* spp., *Rhodotorula mucilaginosa*, *Fusarium verticillioides*, *P. citrinum*, *A. flavus, F. oxysporum*.

Regarding fennel samples, Hashem & Alamri (2010), found in fennel spices, detected the presence of *Alternaria alternata, A. flavus, A. versicolor* and *C. cladosporioides* in the analyzed samples. Ahene *et al.,* (2011) reported the fungal species found in fennel sold in Africa, with the species *A. flavus, A. fumigatus, A. alutaceus, A. niger, A. sulphureus* being isolated. Kulshrestha *et al.,* (2014) found the highest percentages in *Aspergillus flavus* (18.5%).

Also isolating *Alternaria alternata, A. candidus, Cinereus microascus, P. chrysogenum, P. notatum, Rhizopus* and *Trichoderma* spp. In our sample, *A. niger* (18.3%) found the highest percentages, followed by *A.*

ochraceus (15.4%) and finally *A. flavus* (14.4%) (Figure 2).

The most predominant fungal genus found in our research was the Aspergillus genus, represented by 13 species in the samples, with *Aspergillus niger, A. ochraceus, A. flavus* and *A. terreus* being the most representative. Similar results were observed by other researchers in their case series (Syamilah *et al.,* 2022; Nurtjahja *et al.,* 2019; Freire *et al.,* 2000; Klimesová *et al.,* 2015; Al-abas *et al.,* 2020; Ahene *et al.,* 2011; Shiva Rani, 2021; Rani and Saena, 2022) who reported that *Aspergillus flavus, A. fumigatus* and *A. niger* were dominant in their survey of spice microbiota.

It is necessary to raise an alert and draw attention to the isolation of entities of the genus Aspergillus, considered one of the most common isolates in the studies presented in this case series. Current studies report that strains are becoming resistant to the use of antifungals used in clinical treatment. Researchers (Ren *et al.,* 2016; Verweij *et al.,* 2020; Rhodes *et al.,* 2022) showed in their series that strains of *A. fumigatus*, treated with azole pesticides such as tebuconazole, metconazole and propiconazole showed resistance to azoles clinical trials, showing mutations in the *cyp51A* genes, characterizing possible cross-resistance.

Recent research has revealed that strains of *A. flavus*, the second largest cause and one of the main agents of serious comorbidities, including Aspergillosis; also causing co-infections in patients with severe infection by the SARS-CoV-2 virus, leading to pulmonary aspergillosis associated with COVID-19 (Mariscal *et al.,* 2023; Simmons *et al.,* 2023) were tested after exposure to the same azoles agricultural products had the MIC (Minimum Inhibitory Concentration) for drugs of the same class, posaconazole, voriconazole and itraconazole increased, showing that agricultural pesticides can, in fact, reduce the effectiveness of antifungals used in the clinic (Meireles *et al.,* 2019).

Yeast-Form Fungi

In relation to yeast-like fungi, little is said about the studies used as a basis for this. The vast majority of studies refer only to molds and yeasts, which are relegated to the background, without identification of their species. In this study, yeasts represented 8,7% of the total microbial species isolated. The three most isolated yeasts were *R. mucilaginosa, C. krusei* and *S. cerevisiae* found, as well as the other yeasts, in samples of cinnamon sticks, star anise, bay leaves, saffron, calabrian pepper, cloves, cumin, fennel, nutmeg and garlic. It is worth mentioning that *R. mucilaginosa* presented the highest isolation rates (2.4%) among the other yeasts.

Analysis of condiments and spices, carried out by Teixeira-Loyola (2014) identified, in addition to the filamentous fungi *Aspergillus* and *Penicillium*, samples of *C. tropicalis* and *Rhodotorula* spp., in samples of bay leaves, basil, oregano and cloves in the mining city of Pouso Alegre/Brazil.

Turkish researchers found in samples black pepper (70%), cumin (80%), allspice (*Pimenta dioica*) (90%), pepperoni pepper (*Capsicum baccatum*) (90%), red fire pepper (*Capsicum* spp.) (60%), *C. albicans* yeast (1.7 X 10^3) in black pepper and *C. tropicalis* (6.7 X $10^3 \pm 7.3$ in addition to other filamentous fungi such as *A. flavus, A. fumigatus* and *A. niger* (Vural *et al.,* 2004).

Cunha Neto *et al.,* 2013 found *Rhodotorula mucilaginos*a occurred only in black pepper. Other yeasts were less common, occurring in fennel and oregano samples, but were not identified. Kortei *et al.,* (2022a), analyzing herbs and spices in three markets in Ghana (West Africa), found molds in all identified species, with the exception of the *R. mucilaginosa* species being the only yeast among the isolated fungi, with *F. oxysporum* being the most isolated contaminating agent in all the samples.

In microbiological analyzes of preserved green and black olives, Italian researchers (Aponte *et al.,* 2010) found the following yeasts: *C. parapsilosis*, *Pichia guilliermondii* and *P. kluyveri.* Nisiotou *et al.,* (2010) in Greece, found in their series *Metschnikowia pulcherrima*, as the dominant yeast species in fermentation, followed by *Debaryomyces hansenii* and *Aureobasidium pullulans*, in addition to *Candida silvae*, *Cystofilobasidium capitatum*. In 2011, also in Italy, Muccilli *et al.,* (2011) found in the fermentation process of this appetizer, *Saccharomyces cerevisiae, Wickerhamomyces anomalus, C. diddensiae* and *Issatchenkia orientalis* and finally, Spanish researchers in Catalonia (Beraldo *et al.,* 2013), found in the olives, *C. boidinii, C. sorsoba, C diddensiae, P. membranifaciens, P. anomala, P. kluyveri, Kluyveromyces lactis* and *R. glutinis*, during the production and maturation process of the Mediterranean fruit.

Table.1 Isolated fungal and bacterial species, originating from dehydrated aromatic condiments and spices sold in free fairs and supermarkets in the city of Cuiabá-MT, central region of Brazil

	Dried aromatic spices and condiments												
Fungal species	***Cinnamomum verum***	***Syzygium aromaticum***	***Illicim verum***	***Pimpinella anisum***	***Piper nigrum***	***Cuminum cyminum***	***Laurus nobilis***	***Myristica fragans***	***Curcuma longa***	***Zingiber officinale***	***Allium sativum***	***Capsitum baccatum***	***Origanum vulgare***
Absidia corymbifera	-	X	-	-	-	-	-	-	-	-	-	-	-
Alternaria alternata	-	-	-	-	-	-	-	-	-	X	-	X	X
Aspegillus nidulans	X	-	-	-	-	-	-	-	-	-	X	-	-
Aspergillus candidum	-	-	-	-	-	-	-	-	-	-	X	-	-
Aspergillus flavus	X	-	-	X	-	X	X	X	X	-	X	-	X
Aspergillus fumigatus	X	-	-	X	-	-	-	-	-	-	-	X	-
Aspergillus nidulans	-	X	X	-	X	-	-	-	-	-	-	-	-
Aspergillus niger	X	X	X	-	-	X	-	X	X	X	X	X	X
Aspergillus ochraceus	-	X	X	X	X	-	X	X	-	X	X	-	X
Aspergillus oryzae	-	-	-	-	-	-	-	-	-	-	X	-	-
Aspergillus parasiticus	-	-	-	-	-	-	-	-	-	-	-	X	-
Aspergillus sydowii	-	-	-	-	X	-	-	-	-	-	-	-	-
Aspergillus tamarii	-	-	-	-	-	-	-	-	-	X	X	-	X
Aspergillus terreus	X	-	-	-	X	-	-	X	X	X	X	X	-

Aspergillus ustus	-	-	-	-	-	-	-	-	-	-	-	-	X
Candida albicans	-	-	-	-	-	-	X	-	-	-	-	-	-
Candida guilliermondii	-	-	X	-	-	-	-	-	-	-	-	-	-
Candida krusei	X	-	-	X	-	-	-	X	-	-	-	-	-
Candida parapsilosis	X	X	-	-	-	-	-	-	-	-	-	-	-
Candida tropicalis	-	-	-	-	-	X	-	-	-	-	-	-	-
Cladophialophora carrionii	-	-	-	-	-	-	-	-	X	-	-	X	-
Cladosporium cladosporioides	-	X	-	-	-	-	-	-	-	-	X	X	X
Cladosporium herbariorum	X	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium herbarum	-	-	-	-	-	X	-	-	-	X	-	-	-
Cladosporium sphaerospermum	-	-	X	-	-	-	-	-	-	-	-	-	-
***Cryptococcus* spp.**	-	X	-	-	-	-	-	-	-	-	-	-	-
Curvularia clavata	X	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia curvata	-	-	-	-	-	-	-	-	-	-	-	X	-
Escherichia coli	-	-	-	X	-	X	-	-	-	-	-	-	-
Eurotium amstelodami	-	-	X	X	-	-	-	-	-	-	-	-	-
Eurotium herbariorum	X	X	-	-	-	-	-	X	-	-	-	-	-
Eurotium repens	X	-	-	-	-	-	-	-	-	-	-	-	-

Fonsecae pedrosoi	-	-	-	-	-	-	-	-	-	-	-	-	X
Fusarium commune	-	-	-	-	-	-	-	X	-	-	-	-	-
Fusarium oxysporum	X	X	-	-	X	-	X	X	-	-	-	-	-
Fusarium proliferatum	-	-	-	-	-	-	-	X	-	-	-	-	-
Fusarium solani	-	-	X	-	X	-	X	-	-	X	-	-	-
Fusarium verticillioides	X	-	-	-	-	-	-	-	-	-	-	-	-
Mucor hiemalis	-	-	-	-	-	X	-	-	-	-	-	-	-
Nigrospora nigra	-	-	X	-	-	-	-	-	-	-	-	-	-
Paecilomyces variottii	-	-	-	-	-	-	-	-	-	-	X	X	-
Paecilomyces viridis	-	-	-	-	-	-	-	-	-	-	-	X	X
Paeciloyces ramosus	-	-	-	-	-	-	-	-	-	-	X	-	-
Penicilium citrinum	X	-	-	-	-	X	-	-	-	-	-	-	X
Penicillium chrysogenum	-	-	-	-	-	-	-	-	-	-	X	-	-
Penicillium citreonigrum	-	-	-	-	-	X	-	-	-	-	-	-	X
Penicillium citrinum	X	X	-	-	-	X	X	X	-	-	-	X	X
Penicillium commune	-	-	-	-	-	-	-	-	-	-	-	-	X
Penicillium crustosum	-	-	-	-	-	-	-	-	-	-	-	X	-
Penicillium decumbens	-	-	-	-	-	-	-	-	X	-	-	-	-

Penicillium glabrum	-	-	-	-	-	-	-	-	X	X	-	-	-
Penicillium griseofulvum	-	-	-	-	-	-	-	-	-	-	-	X	-
Penicillium italicum	-	-	-	X	-	-	-	-	-	-	-	-	-
Penicillium oxalicum	-	-	-	-	X	-	-	-	-	-	-	-	-
Penicillium purpurogenum	-	-	-	-	-	-	X	-	-	-	-	-	-
Purpureocillium lilacinum	-	-	-	-	-	-	-	-	X	-	X	-	-
Rhizopus arrizhus	-	-	-	X	-	-	-	-	-	-	-	-	-
Rhodotorula glutinis	-	-	-	-	-	-	-	-	-	-	-	X	-
Rhodotorula mucilaginosa	X	-	-	-	-	-	-	-	-	-	X	-	-
Sacharomyces cerevisiae	-	-	-	-	-	-	-	-	X	X	X	-	-
Salmonela gallinarum	-	-	-	-	-	-	X	-	-	-	-	-	-
Scyncephalastrum racemosum	-	-	-	-	-	X	-	-	-	-	-	-	X
Staphylococcus aureus	-	-	-	-	-	-	-	-	X	-	X	X	-
Streptococcus gengivalis	-	-	-	-	-	X	-	-	-	-	-	-	-
Streptococcus mutans	-	-	-	X	-	-	-	-	X	-	-	-	-
***Trichosporon* spp.**	X	-	-	-	-	-	-	-	-	-	-	-	-

Table.2 Mean count of fungi expressed in colony forming unit per gram (CFU/g) of product detected in dried spice samples in fairs of Cuiabá-MT/Brazil.

Spice/Condiments	**Fair 1**		**Fair 2**		**Fair 3**	
	Microrganism	CFU/g	Microrganism	CFU/g	Microrganism	**CFU/g**
Cinnamon sticks (*Cinnamomum verum* J. Presi)	*Eurotion repens*	2.3 x 10^3	*Aspergillus flavus*	2.8 x 10^3	*Aspergillus terreus*	**2.1 x 10^3**
	Curvularia clavata	2.4 x 10^3	*Penicillium citrinum*	3.2 x 10^4	*Cladosporium cladosporioides*	**5.2 x 10^2**
	Candida krusei	2,8 x 10^3	*Aspergillus fumigatus*	2.3 x 10^2	*Candida parapsilosis*	**1.1 x 10^3**
Clove flowers (*Sygygium aromaticum* L.)	*Aspegillus nidulans*	7.5 x 10^4	*Absidia corymbifera*	2.2 x10^2	*Aspergillus niger*	**3.2 x 10^3**
	Fusarium oxysporum	1.4 x 10^3	*Eurotium herbariorum*	3.2 x 10^4	*Aspergillus ochraceus*	**1.7 x 10^3**
	Cladosporium cladosporioides	1.8×10^3	-	-	-	**-**
Star anise (*Illicium verum* Hook. f.)	*Fusarium solani*	3.3 x 10^2	*Aspergillus ochraceus*	2.5 x 10^3	*Aspergillus niger*	**3.5 x 10^3**
	Cladosporium sphaerospermum	3.1 x 10^3	*Candida guilliermondii*	1.52 x 10^3	*Aspergillus nidulans*	**3.9 x 10^3**
	Eurotium amstelodami	1.2 x10^2	-	-	-	**-**
Anise seed (*Pimpinella anisum* L.)	*Penicillium italicum*	1.7 x 10^3	*Aspergillus fumigatus*	6.8 x 10^3	*Aspergillus ochraceus*	**5.6 x 10^3**
	Escherichia coli	2.7 x10^2	*Streptococcus mutans*	1.4 x 10^3	*Eurotium amstelodami*	**2.9 x10^2**
Black pepper (*Piper nigrum* L.),	*Aspergillus nidulans*	6.7 x 10^3	*Aspergillus sydowii*	2.5 x 10^2	*Penicillium oxalicum*	**2.7 x 10^3**
	Aspergillus ochraceus	2.1 x 10^3	*Fusarium oxysporum*	5.23 x 10^2	*Aspergillus terreus*	**3.2 x 10^4**
	-		*Fusarium solani*	7.6 x 10^2	-	
Cumin grains (*Cuminum cyminum* L.)	*Mucor hiemalis*	2.0 x 10^3	*Streptococcus gengivalis*	3.2 x 10^3	*Penicillium crysogenum*	**7.72 x 10^3**
	Aspergillus niger	3.6 x 10^3	*Escherichia coli*	1.5 x 10^3	*Penicillium citrinum*	**4.5 x 10^2**
	Aspergillus flavus	1.7 x 10^3	*Cladosporium cladosporioides*	1.6 x 10^2	-	**-**
	-	-	*Syncephalastrum racemosum*	3.7 x 10^3	-	**-**
Laurel leaves (*Laurus nobilis* L.)	*Penicillium citrinum*	3.9 x 10^3	*Aspergillus flavus*	2.1 x 10^3	*Fusarium solani*	**3.9 x 10^2**
	Penicillium purpurogenum	5.26	*Aspergillus ochraceus*	7.1 x 10^2	*Candida albicans*	**2.7 x 10^3**

		$\times10^2$				
	-	-	*Aspergillus nidulans*	1.39 x 10^2	*Fusarium oxysporum*	**1.4 x 10^3**
	-	-	*Salmonella gallinarum*	1.93 x 10^3	-	**-**
Nutmeg seed (*Myristica fragrans* Houtt.)	*Fusarium commune*	1.7 x 10^3	*Fusarium proliferatum*	5.95 x 10^3	*Aspergillus ochraceus*	**2.3×10^3**
	Aspergillus terreus	3.2 x 10^3	*Fusarium oxysporum*	6.36 x 10^3	*Eurotium herboriorum*	**1.2 x10^2**
	Penicillium citrinum	1.1 x 10^3	*Aspergillus flavus*	2.3 x 10^3	*Aspergillus niger*	**3.6 x 10^3**
Turmeric flakes (*Curcuma longa* L.)	*Penicillium decumbens*	3.9 x 10^3	*Aspergillus terreus*	2.1 x 10^3	*Cladophialophora carrionii*	**2.2 x 10^3**
	Purpureocillium lilacinum	1.89 × 10^2	*Aspergillus flavus*	3.7 x 10^3	*Sacharomyces cerevisiae*	**1.66 x 10^2**
	Staphylococcus aureus	1.9 x 10^3	-	-	*Aspergillus niger*	**1.9 x 10^4**
	Streptococcus mutans	2.5 x 10^3	-	-	-	**-**
Ginger flakes (*Zingiber officinale* Rosc.)	*Sacharomyces cerevisiae*	1.2 x 10^3	*Fusarium solani*	3.8 x 10^2	*Aspergillus terreus*	**8.61 × 10^2**
	Alternaria alternata	2.3 x 10^3	*Aspergillus tamarii*	4.7x10^2	-	**-**
	Aspergillus ochraceus	2.1 x 10^3	*Aspergillus niger*	5.5 x 10^3	-	**-**
Garlic flakes (*Allium sativum* L.)	*Aspegillus nidulans*	1.7 x 10^3	*Purpureocillium lilacinum*	2.8 x 10^3	*Aspergillus terreus*	**1.9 x 10^4**
	Aspergillus niger	8.5 x 10^3	*Penicillium chrysogenum*	5.4 x 10^2	*Aspergillus ochraceus*	**2.0 x 10^3**
	-	-	*Cladosporium cladosporioides*	3.6 x 10^2	*Aspergillus niger*	**1.5 x 10^3**
	-	-	*Paecilomyces ramosus*	1.1 x 10^3	-	**-**
Pepperoni pepper flakes (*Capsicum baccatum* L.)	*Alternaria alternata*	1.6 x 10^3	*Paecilomyces variottii*	3.1 x 10^3	*Cladosporium cladosporioides*	**1.0 × 10^2**
	Aspergillus terreus	4.49 x 10^3	*Aspergillus niger*	2.06 × 10^3	*Aspergillus fumigatus*	**4.29 x 10^3**
	-	-	-	-	*Curvularia curvata*	**2.2 x 10^3**
	-	-	-	-	*Penicillium citrinum*	**1.95 x 10^3**
Oregano leaves (*Origanum vulgare* L.)	*Fonsecae pedrosoi*	2.8 x 10^2	*Penicillium citrinum*	4.7 x 10^2	*Aspergillus niger*	**9.8 x 10^3**
	Alternaria alternata	3.2 x 10^4	*Penicillium citrionigrum*	7.1 x 10^3	*Aspergillus flavus*	**2.2 x 10^3**
	Aspergillus ustus	2.3 x 10^2	*Scyncephalastrum racemosum*	1.1 x 10^3	**-**	**-**

Means are average of triplicate and expressed as CFU/g

Table.3 Mean count of fungi expressed in colony forming unit per gram (CFU/g) of product detected in dried spice samples in supermarkets of Cuiabá-MT/Brazil.

Spice/Condiments	Supermarket 1		Supermarket 2		Supermarket 3	
	Microrganism	CFU/g	Microrganism	CFU/g	Microrganism	CFU/g
Cinnamon sticks (*Cinnamomum verum* J. Prezi)	*Curvularia clavata*	1.4×10^3	*Aspergillus niger*	2.3×10^3	*Penicillium citrinum*	**5.2×10^4**
	Trichosporon spp.	1.1×10^3	*Fusarium verticillioides*	1.7×10^2	*Aspergillus flavus*	**6.5×10^3**
	Rhodotorula mucilaginosa	2.11×10^3	-	-	*Fusarium oxysporum*	**3.3×10^3**
	-	-	-	-	*Aspergillus terreus*	**6.2×10^3**
Clove flowers (*Sygygium aromaticum* L.)	*Aspergillus nidulans*	3.1×10^3	*Aspergillus niger*	3.1×10^3	*Candida parapsilosis*	**2.1×10^3**
	Fusarium oxysporum	1.95×10^3	*Aspergillus ochraceus*	3.08×10^3	*Cryptococcus* spp.	**1.5×10^3**
	Penicillium citrinum	1.7×10^2	*Cladosporium herbariorum*	2.3×10^2	-	**-**
Star anise (*Illicium verum* Hook. f.)	*Aspergillus niger*	5.3×10^3	*Fusarium solani*	2.2×10^2	*Aspergillus terreus*	**2.3×10^2**
	Aspergillus nidulans	1.2×10^4	*Aspergillus fumigatus*	2.1×10^3	*Nigrospora nigra*	**1.5×10^3**
	Aspergillus ochraceus	5.8×10^3	-	-	*Penicillium citrinum*	**1.5×10^3**
	Eurotium amstelodami	1.2×10^2	-	-	-	**-**
Fennel seed (*Pimpinella anisum* L.)	*Fusarium oxysporum*	2.3×10^3	*Candida krusei*	2.4×10^3	*Aspergillus niger*	**$3{,}2 \times 10^4$**
	Aspergillus ochraceus	3.5×10^2	*Fusarium verticillioides*	3.5×10^3	*Mucor hiemalis*	**$2{,}5 \times 10^3$**
	-	-	*Rhizopus arrizhus*	2.8×10^3	-	
Black pepper (*Piper nigrum* L.),	*Aspergillus niger*	1.9×10^3	*Cladosporium herbariorum*	3.3×10^3	*Penicillium decumbens*	**$2{,}5 \times 10^3$**
	Eurotium amstelodami	2.5×10^2	*Penicillium citreonigrum*	2.2×10^3	*Aspergillus ochraceus*	**$6{,}9 \times 10^3$**
	Aspergillus flavus	1.2×10^3	*Aspergillus flavus*	1.95×10^3	-	
Cumin grains (*Cuminum cyminum* L.)	*Fusarium proliferatum*	2.3×10^3	*Eurotium herbariorum*	3.6×10^2	*Mucor hiemalis*	**$3{,}2 \times 10^3$**
	Penicillium citrinum	2.1×10^3	*Candida tropicalis*	8.5×10^2	*Aspergillus ochraceus*	**$2{,}3 \times 10^3$**
	Aspergillus flavus	1.7×10^3	*Penicillium decumbens*	2.5×10^3	*Aspergillus terreus*	**$2{,}1 \times 10^3$**
	-	-	*Aspergillus niger*	$3{,}5 \times 10^3$	-	
Laurel leaves (*Laurus nobilis* L.)	*Penicillium decumbens*	1.3×10^2	*Fusarium proliferatum*	1.8×10^3	*Aspergillus niger*	**$3{,}5 \times 10^3$**
	Fusarium oxysporum	2.7×10^3	*Aspergillus flavus*	2.8×10^3	*Curvularia clavata*	**$2{,}1 \times 10^3$**

	Aspergillus ochraceus	3.2 x 10^3	*Mucor hiemalis*	5.2 x 10^2	*Aspergillus terreus*	**4,4 x 10^3**
Nutmeg seed (*Myristica fragrans* Houtt.)	*Candida krusei*	1.5 x 10^3	*Aspergillus niger*	2.3 x 10^3	*Aspergillus nidulans*	**1,7 x 10^3**
	Paecilomyces variotti	5.21 x 10^3	*Cladosporium cladosporioides*	2.2 x 10^2	-	**-**
	Rodothorula glutinis	3.5 x 10^3	-	-	-	**-**
Turmeric flakes (*Curcuma longa* L.)	*Fusarium commune*	8.52 x 10^2	*Rodothorula mucilaginosa*	1.2 x 10^3	*Aspergillus parasiticus*	**2,3 x 10^3**
	Purpureocillium lilacinum	3.1 x 10^3	*Aspergillus niger*	8.2 x 10^4	*Curvularia curvata*	**1,7 x 10^2**
	Cladosporium herbarum	2.6 x 10^3	*Penicillium glabrum*	2.7 x 10^3	-	**-**
Ginger flakes (*Zingiber officinale* Rosc.)	*Cladosporium herbarum*	3.3 x 10^3	*Purpureocillium lilacinum*	3.4 x 10^3	*Cladosporium herbarum*	**2,9 x 10^3**
	Aspergillus nidulans	1.2 x 10^2	*Aspergillus tamarii*	2.4 x 10^3	*Rodothorula mucilaginosa*	**2,2 x 10^3**
	Penicillium griseofulvum	3.5 x 10^3	*Penicillium chrysogenum*	2.2 x 10^4	-	**-**
	-	-	*Penicillium glabrum*	2.9 x 10^3	-	**-**
Garlic flakes (*Allium sativum* L.)	*Sacharomyces cerevisiae*	2.1 x 10^3	*Rodothorula mucilaginosa*	7.2 x 10^2	*Paecilomyces variottii*	**3,5 x 10^3**
	Aspergillus flavus	1.4 x 10^3	*Staphylococcus aureus*	2.4 x 10^3	*Aspergillus ochraceus*	**7,1 x 10^2**
	Aspergillus tamarii	3.4 x 10^3	-	-	*Aspergillus oryzae*	**5 x 10^3**
	Cladosporium cladosporioides	1.6 x 10^2	-	-	-	**-**
Pepperoni pepper flakes (*Capsicum baccatum* L.)	*Cladophialophora carrionii*	2.06 × 10^3	*Rodothorula glutinis*	2.5 x 10^3	*Paecilomyces variottii*	**3,4 x 10^3**
	Penicillium candidum	2.1 x 10^3	*Aspergillus parasiticum*	1.5 x 10^2	*Aspergillus terreus*	**5,1 x 10^3**
	Penicillium crustosum	1.6 x 10^3	*Staphylococcus aureus*	2.1 x 10^3	*Penicillium griseofulvum*	**1,3 x 10^3**
	-	-	-	-	*Aspergillus fumigatus*	**3,1 x 10^4**
Oregano leaves (*Origanum vulgare* L.)	*Penicillium commune*	1.3 x 10^3	*Aspergillus niger*	5.4 x 10^3	*Cladosporium cladosporioides*	**4,6 x 10^2**
	Paecilomyces viridis	3.8 x 10^2	*Penicillium citrinum*	2.6 x 10^2	*Aspergillus flavus*	**2,1 x 10^3**
	Aspergillus tamarii	**2.4 x 10^2**	**-**	**-**	***Aspergillus ochraceus***	**1,6 x 10^2**

Means are average of triplicate and expressed as CFU/g.

Figure.1 Some fungal species isolated from samples of seasonings and aromatic spices from the city of Cuiabá/MT, central region of Brazil.

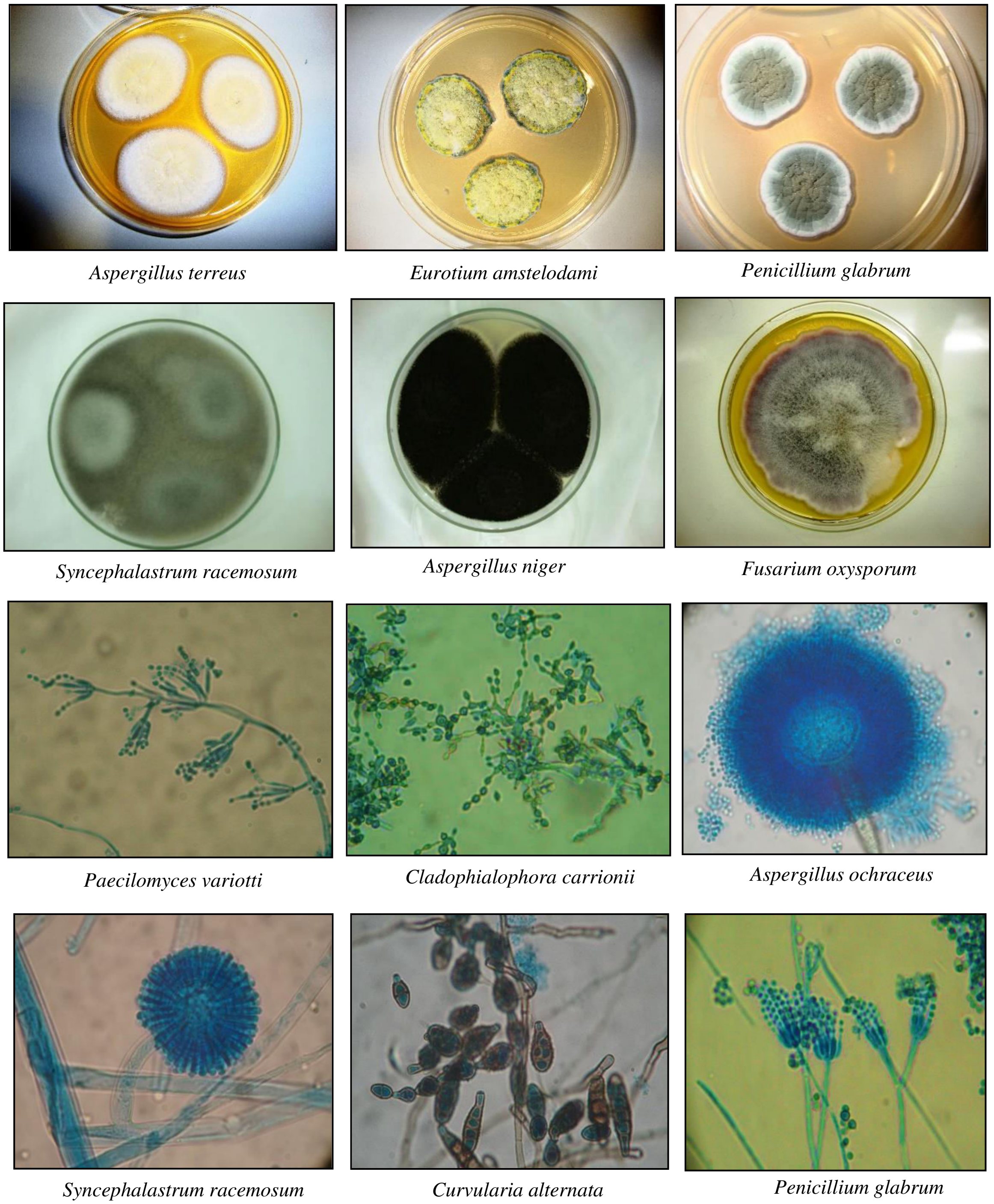
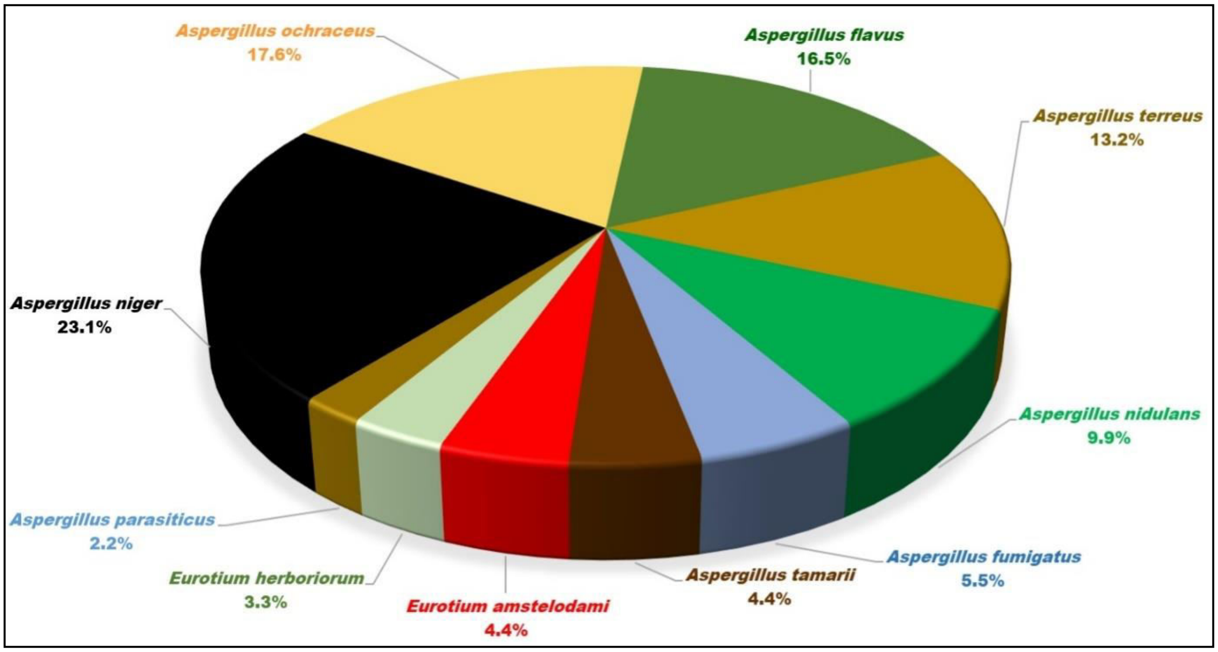


Figure.2 Frequency of the ten most prevalent fungal species in isolates from seasonings and dry condiments sold in free fairs and supermarkets in the city of Cuiabá-MT/Brazil.



An Antimicrobial Action

Interestingly, some studies have reported the power of some spices, herbs and condiments and their extracts, being widely studied as potential antimicrobial agents, which may be important for the preservation of food and the control of human diseases of microbial origin. Researchers from Malaysia reported that in analyzes of fungi in condiments and spices, star anise and cloves were free from fungal contamination (Syamilah *et al.,* 2022).

In 2013, Brazilian researchers evaluated the efficiency of cinnamon and clove essential oils as sanitizers in the food industry, and observed that these oils showed inhibition on bacteria *S. aureus, E. coli, Campylobacter jejuni, S. enteritidis, Listeria monocytogenes* and *S. epidermides* (Beraldo *et al.,* 2013). In 2012, other Brazilian researchers tested the properties of these essential oils, found against fungi isolated from dermatomycosis, such as *C. albicans, Trichophyton mentagrophytes, Saccharomyces cerevisiae* and *A. niger* (Affonso *et al.,* 2012) with around 80-90% specificity.

Walker *et al.,* (2014), analyzing several condiments, found no records of contamination by fungi and bacteria in garlic and oregano. Cinnamon extract demonstrated antifungal potential, indicating the possibility of its use in the in vitro control of phytopathogenic fungi such as Alternaria alternata and Fusarium oxyporum, tested by Carmello *et al.,* (2022).

Antimicrobial action of spices on bacterial development was tested by Binatti *et al.,* (2016) using star anise, cinnamon bark, cardamom, cloves, fennel and pepper to control bacteria of the genera *Staphylococcus, Salmonella* and *Bacillus*, where the best results were shown for pungente aromatic cloves.

However, other Brazilian researchers (Santoro *et al.,* 2007) tested the active ingredients, eugenol, the main constituent of the oil (86.34%) against forms of the protozoa *Trypanosoma cruzi*, which proved susceptible to treatment. Epimastigote forms of *Leishmania amazonensis* were also tested, resulting in 100% mortality of the protozoa. Azeredo and Soares (2013) analyzed the effect of the combination of citral, eugenol and thymol, constituents of the essential oils of *Cympobogon citratus* (lemongrass), *Syzygium aromaticum* (clove) and *Thymus vulgaris* (thyme), on the proliferation of the trypanosomatids *Crithidia fasciculata* and *Trypanosoma cruzi.*

Brazil is the country most affected by Dengue; in 2020, researchers from Alagoas evaluated cloves, cinnamon and garlic, and verified their larvicidal action against *Aedes aegypti*, as a natural alternative for combating these arthropods (Palmeira *et al.,* 2020).

Bacterial Isolation

Regarding isolated bacteria, such as *Streptococcus mutans* (1.9%) and *S. gengivalis* (1.0%), these contaminating bacteria are found as part of the oral microbiota of humans and animals. The findings of these oral microorganisms, in this research, indicate that their presence is due to the dispersion of spittles expelled through the mouth during the conversation process during the purchase and sale of products, carried out between customers and stallholders, promoting contamination in samples of anise, seeds cumin and turmeric, where these specimens were isolated.

The climatic conditions found in the Central region of Brazil, as well as in other tropical and subtropical countries, favor the growth of filamentous fungi of the genus Aspergillus, according to reports (Pitt and Hocking, 1999). Kabak & Dobson (2017) describe that spices and herbs grown mainly in tropical and subtropical areas can be exposed to contamination with toxigenic fungi and subsequently with mycotoxins. In our series, in the Central region of Brazil, Mato Grosso, this characterization is quite consistent with the physiognomic characterization presented by these researchers.

Studies carried out by Martins *et al.,* (2010), showed that industrialized spices from supermarkets, despite strict quality control, showed microbial proliferation for the total count of bacteria, fecal coliforms, molds and yeasts. This researcher and his collaborators found the presence of *E. coli* in samples of cloves, bay leaves, basil and oregano. This bacteria is known to cause organic damage when consumed. In our series, *Escherichia coli* was isolated from fennel samples ($2.7x10^2$ CFU/g) and cumin seeds ($1.5x10^3$ CFU/g), showing the high presence of these bacterial microorganisms in samples of condiments and spices (Table 1). *Staphylococcus aureus* was isolated in samples of turmeric, garlic and pepperoni pepper (Table 2 and 3).

This bacterium is the most dangerous among all staphylococcal bacteria, as its presence causes skin infections, pneumonia, endocarditis and osteomyelitis (Santos *et al.,* 2007). About 30% of healthy people carry the bacteria in their nostrils and 20% on the surface of their skin. Research data from the US National Hospital Infection Surveillance (NNIS) reports that this species has acquired easy resistance, exceeding 50% in ICUs in that country. In Brazilian hospitals, this rate varies between 40% and 80% (Santos *et al.,* 2007). In this study these bacterial microorganisms were the most isolated.

Another bacterial microorganism found was Salmonella gallinarum, the causative agent of fowl typhus. The genus Salmonella is a bacterium originating from two species (*S. enterica* and *S. bongori*) and several pathogenic serovars such as: *Enteritidis, Typhimurium, Gallinarum* and *Pullorum*, which are contaminants of poultry-derived foods (Brasil, 2020), highly adapted to the man. Salmonella of enteric origin, of greatest relevance to public health, are composed of six subspecies (*S. enterica, S. salamae, S. arizonae, S. diarizonae, S. houtenae, S. indica*) (Jahan *et al.,* 2022).

The insertion of this bacterial specimen is due to the presence of pigeons and their excreta, present in the collection sites, especially in street markets, where these birds live in search of food and live in synanthropy with humans, promoting contamination of food displayed on countertops, during its flights through the environment.

Transmission occurs through ingestion of food contaminated with animal feces. The bacteria is normally found in animals such as chickens, pigs, reptiles, amphibians, cows and even in domestic animals, such as dogs and cats (Brasil, 2020).

These serovars, called zoonotic Salmonella, can affect humans and animals alike, which are responsible for gastroenteritis (enterocolitis) or foodborne diseases.

According to the Epidemiological Bulletin of the Brazilian Ministry of Health in 2020 (Brasil, 2020) on outbreaks reported in water and food, the most prevalent agents were *Escherichia coli* (35.7%), *Salmonella* (14.9%), *Staphyococcus* (11.5%), *Norovirus* (8.3%), *Bacillus cereus* (7.4%) and *Rotavirus* (6.95%).

In 2015 (Klimesová *et al.,* 2015), in research on condiments *Bacillus cereus* was present in juniper, mustard, bay leaves, thyme and cardamom (32%), while *Bacillus lichniformes* was isolated in 58% in allspice, red pepper, juniper, bay leaf, paprika, garlic, thyme and cardamom. *Staphylococcus aureus*, isolated from coriander (30%), cinnamon (40%), badiana (20%) and mustard (10%). The presence of *Salmonella* spp and *E. coli* has not been confirmed.

Research carried out in the city of Barra do Garças, in Mato Grosso, Central region of Brazil (Oliveira *et al.,* 2023) researchers from that city, found specimens of *Salmonella* spp. in saffron (*Curcuma longa*) and black pepper (*Piper nigrum*) samples, 60% of black pepper samples showed *Staphylococcus aureus* counts and 66.67% for saffron. Silva *et al.,* (2013) recorded the presence of *S. aureus* in 86% of the black pepper samples analyzed and the abundant presence of *E. coli* in the black pepper and cumin condiments.

However, Furlaneto & Mendes (Ahene *et al.,* 2011), in their series, attested to the absence of this enteropathogen in dehydrated oregano, parsley and basil, as well as Veloso *et al.,* (2022), after the analyses, were able to observe that the absence of *Salmonella* spp., *S. aureus* and *E. coli*, in samples of coloriferous (annatto), oregano and black pepper. In Palmas, Tocantins (Walker *et al.,* 2014), Amazon region of Brazil, absence was found in garlic and oregano samples.

Ogur (2022) in Turkey, determining the microbiological quality and safety of spices, found *E. coli, S. aureus* and *Salmonella* spp. in red pepper flakes, black pepper, dried mint, dried thyme and ground cumin. 62.96% of the samples contained a group of fecal coliforms and 46.29% of the samples showed unacceptable limits of *E. coli* (87.03%) and *Salmonella* spp. (38.90%). Dried thyme showed the best microbiological quality.

The studies presented show results that demonstrate that the presence of potentially pathogenic and spoilage microorganisms is indicative of inadequate hygienic-sanitary procedures and results in loads of pathogenic microorganisms in the dried spices analyzed, presenting high levels and, therefore, unsafe in terms of microbiological quality.

According to the data presented, it is concluded that condiments sold in free fairs and industrialized condiments sold in supermarkets are contaminated with potentially pathogenic fungi that produce mycotoxins.

These results corroborate other published articles presenting similar data, raising a worrying question about the hygiene of handlers, utensils and equipment used in the manufacture of the product, as well as the storage conditions of the condiments and spices evaluated. Therefore, the condiments analyzed here, in our point of view, represent a risk to consumer health, since species isolated in spices are potentially pathogenic.

Therefore, in addition to the need to inform consumers about the risk of consuming products contaminated with fungi, it would be of great value to minimize these risks by carrying out training programs for producers on appropriate techniques for handling, drying and storing condiments.

The high counts of fungi found in this study deserve attention, as they can produce mycotoxins, in addition to being able to accelerate the deterioration of food, given their large enzymatic arsenal. The isolation of molds and yeasts is used as an indication of the precarious conditions of food processing and storage operations.

The evident finding of the presence of potentially pathogenic and spoilage microorganisms clearly demonstrates that this is an indication of inadequate hygienic and sanitary procedures throughout the marketing chain, making it necessary to establish rules and guidelines for producers and traders to adopt good agro-agicultura practices, manufacturing and marketing, aiming to obtain a safe and quality product.

In view of the above, it is necessary to emphasize the need for inspection in quality control, at all stages of obtaining condiments and spices, from collection to offering to the consumer, since the levels of fungal contamination found here exceeded the established values by ANVISA (Brasil, 2022), in addition to the possibility that these fungi produce mycotoxins.

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Author Contributions

DPLJ (leader) conceived, contributed to the design of the study, data analysis, interpreted the results, reviewed and edited the manuscript. VKVO wrote the manuscript, read and revised and interpreted the document. AAGN, BOL, BMS, MLM, CCP and MLSM collected the data, organized the information and assisted in checking the manuscript. All authors read and approved the final version of the manuscript. All authors had full access to all study data and had final responsibility for the decision to submit for publication.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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