Microbiological analysis of peanuts commercialized in the city of São José dos Campos - SP in Brazil

Análise microbiológica de amendoim comercializado na cidade de São José dos Campos - SP no Brasil

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ABSTRACT
Through microbiological analysis in peanuts sold in the city of São José dos campos - SP; Brazil, the presence of Aspergillus flavus was verified through growth in PDA Agar Dextrose and Sabouraud Dextrose culture media. Peanuts were collected in bulk, with two samples from all different points of trade about 50g per sample, totaling 13 points collected in the city of São José dos Campos - SP; Brazil, (totaling 26 samples collected). The samples were separated into groups (group A with 13 samples), (Group B with 13 samples), soon the (group A), were seeded in PDA Dextrose acidified culture medium ph 6.5 and group B in Sabouraud Dextrose culture medium, soon they were incubated for seven days at 30º C temperature. We obtained as results the culture media positive in two samples of (group A) and two samples in (group B), thus we observed the growth of Aspergillus flavus. Thus, we concluded that four peanut sales points commercialized in the city of São José dos Campos contained samples contaminated by Aspergillus flavus fungi, presenting a great risk of toxicological and pathological contamination to consumers of this product.

Keywords: Aspergillus flavus, aflatoxins, peanut.

RESUMO
Por meio de análise microbiológica em amendoins comercializados na cidade de São José dos campos - SP; Brasil, verificou-se a presença de Aspergillus flavus através do crescimento em meios de cultura PDA Agar Dextrose e Sabouraud Dextrose. Os amendoins foram coletados a granel, com duas amostras de todos os diferentes pontos de comércio, cerca de 50g por amostra, totalizando 13 pontos coletados na cidade de São José dos Campos - SP; Brasil, (totalizando 26 amostras coletadas). As amostras foram separadas em grupos (grupo A com 13 amostras), (grupo
B com 13 amostras), logo o (grupo A), foram semeadas em meio de cultura PDA Dextrose acidificada ph 6,5 e o grupo B em meio de cultura Sabouraud Dextrose, logo foram incubadas por sete dias a 30º C de temperatura. Obtivemos como resultados os meios de cultura positivos em duas amostras do (grupo A) e duas amostras do (grupo B), assim observamos o crescimento de Aspergillus flavus. Dessa forma, concluímos que quatro pontos de venda de amendoim comercializados na cidade de São José dos Campos continham amostras contaminadas pelo fungo Aspergillus flavus, apresentando um grande risco de contaminação toxicológica e patológica aos consumidores desse produto.

**Palavras-chave:** Aspergillus flavus, aflatoxinas, amendoim.

### 1 INTRODUCTION

Benefiting human beings, peanuts today are a food consumed very frequently by Brazilians and spices worldwide, being employed as a food that is part of our culture, in typical cuisines, snacks, sweets, oils and butters in general.[1] Considering the need of our population to enrich the quality of the diet, the grains are presented as a beneficial food for the cardiovascular system, because they present in their composition phytosteroids, which contribute to the prevention of cardiovascular diseases such as atherosclerosis, this contribution is attributed to the increase of good cholesterol (HDL), besides presenting tocopherol and B-carotene, substances that present other beneficial effects to health. Along with these benefits spice grains is a compound rich in high amount of essential amino acids, important minerals and essential salts. [2]

The great concern is that the peanut grain is considered an oil seed, as classified by ANVISA [3]. Thus it presents a great possibility of rapid colonization by Aspergillus flavus fungi, this can cause great toxicological and pathological impact to humans and animals. These fungi are located preferentially in the soil, air and in the food itself, hospitals in immunosuppressed patients and in the oil-rich grains themselves, in this situation of colonization and primary source of contamination to humans, can be aggravated by the use of inadequate agricultural practices in the drying stage and bad storage of producers and stores, in the incorrect handling of food, then the Aspergillus flavus are fungi that produce "Aflatoxin B1", a mycotoxin highly carcinogenic to humans. [4,5,6]

Aflatoxin B1 can trigger impacting pathologies in the individual, leading to acute or chronic poisoning, among the symptoms are cited nausea, fever, fatigue, pain in the abdominal region, lack of appetite, with the possibility of worsening since other pictures that may be present...
in a contaminated individual aggravating to hepatic necrosis and chronic renal failure, myocarditis, pulmonary effisema, hepatic hemorrhage, gastric hemorrhage, jaundice, lethargy and even death. [7,8]

Thus the objective of the present work was to evaluate the presence of Aspergillus flavus in peanut samples commercialized in the municipality of São José dos Campos - SP; Brazil, as well as to alert about the great risks of this fungal infection.

2 MATERIAL AND METHODS
2.1 SAMPLE COLLECTION AND SCREENING

For the proposed study, about 26 samples of peanut kernels in natura were collected, about 50g of peanuts in each sample in sterile tubes, being collected in the main points of commerce of spices in bulk in the city, in each point of collection 2 samples were acquired in duplicate from the 13 commercial establishments, then they were divided into (Group A = 13 samples) and (Group B = 13 samples) and numbered 1 to 13 for each group

Figure 1. Samples (A) after separate collection in groups (A and B) in sterile field in the laminar hood.

2.2 CULTURES OF THE SAMPLES SOWING CONDITIONING IN GREENHOUSES AND PROCEDURES

The peanut samples were conditioned at room temperature, the samples collected were analyzed in August 2021, through the technique of direct plating on Petri plates, containing the culture medium Agar potato dextrose (PDA) acidified Ph 6.5 for the (group A); Each plate contained 5 peanut kernels, cross-seeded and inserted with the help of sterilized tweezers, and then the "positive and negative” controls of AATT Aspergillus flavus strains were
2.3 EVALUATION OF THE SAMPLES IN CULTURE MEDIA AND MICROSCOPIC ANALYSIS

Macroscopic evaluations were performed in the culture media, after which the fungus growth was evaluated and the positive plates for growth were identified, where the slides were made to confirm the presence of A. flavus; In microscopy then were used the materials for assistance as: slide, coverslip and a drop of the dye lactophenol aman blue, then soon were performed the transfers of the colonies of positive culture media for microscopy slides, then they were stained with lactophenol aman blue and evaluated in optical microscopy, for positive confirmations of the growth of Aspergillus flavus. [10]

3 RESULTS

Performed. Next, incubations were performed in bacteriological greenhouses at a temperature of 30°C, for a period of seven days with humidity inside the greenhouses in a beaker with 7 ml of filtered water. [9]

3.1 ANALYSIS OF POSITIVE AND NEGATIVE CULTURES AND MICROSCOPY FOR ASPERGILLUS FLAVUS

Of the 26 samples collected, 4 samples obtained "Positive" results, where they were evaluated by qualitative, positive and negative methods, where we obtained the presence of Aspergillus flavus colonies in the positive samples, after confirmation by light microscopy. In
Figure 1. c, d, e, f; the culture media for positive samples is shown; Figure 2. G, h, i, j; Confirmation of colonies of positive samples by optical microscopy shown in the figures below:

Figure 3. (c), above the on the left side "Control positive" cepa A.flavus ATCC (9743) for PDA dextrose culture medium; (d) on the right side "Control positive" for Sabouraund dextrose culture medium; (e) Sample in positive in PDA dextrose; (f) Sample positive in Sabouraund dextrose culture medium.


Figure 4. (g) Control PDA dextrose sample and sample (3A) positive; (h) Control Sabouraund dextrose sample and sample (4B) positive; (i) Sample (10B) positive; (j) Sample (11A) positive samples by light microscopy; (k) In this picture of the authors themselves taken from the microscopy under evaluation, the whole morphology of the Aspergillus flavus fungus is demonstrated.


The fungus *Aspergillus flavus* has septate, hyaline hyphae, with dichotomous branching forming 45° angles, where the asexual reproduction structures (conidia) are present, located at the top of a terminal vesicle that arises from the extension of the conidiophore where the toxin
called Aflatoxin B1 is released. Knowing that the conidia have the function of detaching themselves from the Phialides and Vesicles to release the small spherical microbubbles full of toxins called Aflatoxin B1, this vesicle is covered by one or two layers of specialized cells and conidia, which are formed asexually and in interconnected chains. These cells that form the conidia are called conidiogenous cells or phialides, so it is called uniseriate Aspergillus flavus. If there is a second layer of cells connecting the phialide to the vesicle, the Aspergillus is referred to as biseriate.¹¹¹

Table 1 shows the groups collected and the results obtained in culture media and *A. flavus* confirmation by light microscopy for the positive and negative samples.

Table 1. found in Fungi peanut samples collected in the municipality of São José dos Campos - SP.

<table>
<thead>
<tr>
<th>Samples collected (Group A, Group B)</th>
<th>Agar Sabouraud (Group A)</th>
<th>Agar PDA Dextrose (Group B)</th>
<th>Lactofenol aman blue staining (Microscopy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controle Positivo <em>A. flavus</em></td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Controle Negativo <em>A. flavus</em></td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
<td>Simples (1A, 1B)</td>
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<td>(-)</td>
<td>(-)</td>
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<tr>
<td>Simples (2A, 2B)</td>
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<td>(-)</td>
<td>(-)</td>
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<tr>
<td>Simples (3A, 3B)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
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<tr>
<td>Simples (4A, 4B)</td>
<td>(-)</td>
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<td>(+)</td>
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<tr>
<td>Simples (5A, 5B)</td>
<td>(-)</td>
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<tr>
<td>Simples (6A, 6B)</td>
<td>(-)</td>
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<tr>
<td>Simples (7A, 7B)</td>
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<tr>
<td>Samples (8A, 8B)</td>
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<td>Samples (9A, 9B)</td>
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<td>Samples (10A, 10B)</td>
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<tr>
<td>Samples (11A, 11B)</td>
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<tr>
<td>Samples (12A, 12B)</td>
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<td>(-)</td>
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<tr>
<td>Samples (13A, 13B)</td>
<td>(-)</td>
<td>(-)</td>
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</tr>
</tbody>
</table>

Total positive samples: 2 2 = 4

Source: Laboratório da Universidade Paulista - UNIP (SJ. Campos), author's own; 2023

3.2 GROWTH PARAMETERS OF THE PRESENT POSITIVE SAMPLES COMPARED TO THE CONTROL SAMPLES

In this evaluation we called the growth criteria in CFU/ml x 105 to monitor which culture medium was most effective in growing *A. flavus* during the positive presences compared to the positive and negative controls shown in figure 5 below:
4 DISCUSSION

The presence of positive samples in the peanut commerce in the city of São José dos Campos - SP; Brazil, alerts and opens the discussion about the possible deficiencies in inspections, a fact that can favor the inadequate handling of food and the development of fungal colonies, which is aggravated by the allowed possibility of dissemination and contamination of the consuming society. It is worth mentioning that the value stipulated for peanut contamination with aflatoxin B1 has already been established by the National Health Surveillance Agency, where they have been further studied and quantified about <20ug/kg allowed per portion of 50g, and for food grains no colony should be present as a reference should be < 1 CFU/ml x 105, and above >1 CFU/ml is considered the presence of fungal contamination, in this same line of microbiological clinical mycological and toxicological research. [12] What allows increasing the inspection in general, so in this present work we obtained "4 positive samples", reinforcing even more this line of discussion, these samples are still being commercialized in a city considered with good technological contribution in the interior of São Paulo - SP in Brazil, in this major problem to be discussed is not only the presence of fungi in the samples, but the release of aflatoxins B1 by these fungi, which contain a highly neoplastic power for humans, in this present work we researched in depth the identification and presence of A. flavus in the samples, evaluating the quantities in positive samples by an expressive qualitative method, carried out keeping all the integrity of the samples and asepsis of the media in a laminar flow chapel.[13]
The real purpose of using two culture media "Sabouraud dextrose" and "PDA dextrose" was to verify which of the two culture media would have the best adaptation and growth of the *A. flavus* fungus in the culture media, where we concluded that the two culture media in the positive samples obtained constant development for both types of culture media. We affirm in this research that it is possible to find in the literature the description that aflatoxin B1 is one of the most toxic substances ever produced by microscopic beings, thus aflatoxin B1 intoxication in humans through the ingestion of food contaminated by the fungus *Aspergillus flavus* has the same toxicological power similar to that of a snake, having the potential to cause serious harm to the Brazilian and world population, inducing clinical pictures of high complexity, with prevalence of kidney and liver disorders, besides the possibility of teratogenic effects, as well as carcinogenic effects in the real possibility of death. This fact reinforces the discussion Most people breathe Aspergillus flavus spores every day. However, people with weakened immune systems or lung diseases are at greater risk of developing health problems from these fungi. For example, recent studies in patients ventilated with COVID-19 have reported a higher incidence of aspergillosis, affecting up to 30% of intubated patients. According to the U.S. Centers for Disease Control and Prevention (CDC), the number of aspergillosis-related hospitalizations in the United States increased by an average of 3% per year during 2010-2021. Knowing further that nearly 15,000 hospitalizations associated with aspergillosis occurred in the United States in 2014, at an estimated cost of $1.2 billion. The real contamination from the consumption of these spices, obtained from the commerce of the city of São José dos Campos - SP in Brazil, this present study raises the attention of all patients. In this way, this work reaches its objective of demonstrating the real contamination by the consumption of these spices, obtained from the commerce of São José dos Campos - SP in Brazil, this present study frivolously awakens the humanity of the whole world, to perceive the risk that this contamination brings to humanity, as well as induce the discussion and emphasize the necessity of a bigger performance of the pertinent organs of inspection acting all over the world in all the countries, The results of this research call for further studies to research the creation of anti-fungal drugs, derived from homeopathic organic matter, in the action against anti Aspergillus flavus, in the anti-fungal requirement, due to the scarcity of specific anti-fungals for these fungi called Aspergillus flavus."[14, 15]
5 CONCLUSION

We conclude that the possibility of contamination by Aspergillus flavus fungus through the consumption of peanut kernels commercialized in São José dos Campos - SP in Brazil is real. Thus, the samples in 50g portions showed great "Positivation / Presence" for these toxicological fungi called Aspergillus flavus. In this context, we conclude that the great need for greater supervision, control and guidance of the general population is necessary to increase the safety of the world population in other countries that consume the food grains in the global requirement. It is worth noting the great significance and importance of further research into the studies of the possible creation of antifungals, anti Aspergillus flavus, derived from homeopathic materials for the elimination of these pathological fungi.

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