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Preliminary identification of aflatoxin-producing *A. flavus* Link and *A. parasiticus* Speare in samples of five varieties of dried chili peppers marketed in the city of Oaxaca, Mexico

*Vorläufige Identifizierung der Aflatoxin produzierenden *A. flavus* Link und *A. parasiticus* Speare in Proben von fünf Sorten getrockneter Chilischoten, die in der Stadt Oaxaca, Mexiko, vermarktet werden*

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Summary

Chili is considered one of the most important vegetables worldwide, because of its high profitability and its versatility that allows different foods to be made. During their growth, storage, and commercialization, they can be contaminated mainly by fungi that can be harmful mycotoxin producers for those who consume them. The objective of this work was to identify the fungi *Aspergillus flavus* and *A. parasiticus* in fourteen samples of dried chili peppers *Capsicum annuum* ("guajillo", "ancho", and "de árbol") that were acquired in the main markets in Oaxaca city. To the samples, the moisture percentage was determined in addition to the mold and yeast count; as a result, the identification of the genera of the isolated strains was obtained, estimating the aflatoxin production of the identified *Aspergillus* strains. The fourteen shows exceeded humidity limits and had high fungal contamination, of which six genera were identified, finding a strain of *Aspergillus flavus* and one of *A. parasiticus*. In the aflatoxin production test, both strains showed fluorescence in the coco-agar medium, indicating that they are aflatoxin producers.

Keywords: Mycotoxins, dried chilies, *Aspergillus flavus*, *Aspergillus parasiticus*, aflatoxins

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Introduction

The worldwide area planted with chili amounts to 1.7 million hectares and produces more than 29 million tons (García-Gaytán et al 2017). Mexico ranks second in the cultivated area and produces red chili peppers. In 2016, chili was the country's second most important horticultural crop after tomatoes (SIAP, 2023). The importance of chili cultivation in Mexico is evident both for its wide distribution and for its vast consumption in the country, in addition to being of a comprehensive environmental range, which allows its production throughout the year, thus satisfying the demand for the product in the main cities (Rosas-Contreras et al 2015). Oaxaca is the third largest producer of jalapeño peppers in Mexico, one of the most consumed peppers in the country (Sánchez-Toledano et al 2023). Mexicans' annual per capita consumption of this vegetable is 16 kg yearly (Sahar et al. 2015); it is widely consumed as a main dish, condiment, pickle, and salad (Swamy, 2023). Like all crops, chili is susceptible to damage from biotic and abiotic diseases at any stage of its development (SIAP, 2023). Fungi, bacteria, nematodes, and viruses cause biotic diseases. Abiotic or noninfectious diseases are caused by external factors such as temperature, light, soil moisture, and nutritional imbalance (Abarca, 2000; Méndez-Espinoza and Vallejo, 2019). The genera of pathogenic or contaminating fungi most frequently found in chili peppers are *Alternaria* spp, *Penicillium* spp, *Rhizopus* spp, *Stemphyllium* spp y *Helminthosporium* spp., *Pythium* spp, *Phytophthora capsici*, *Fusarium oxysporium*, *Cercospora capsici*, *Verticillium wilt* sp, *Rhizoctonia solani*, *Leveillula taurica*, *Colletotrichum capsici* (Hussain and Abid, 2011; Costa et al 2019) *Colletostothrichum capsici*, *Aspergillus niger* y *A. flavus* (Chigoziri and Ekefan, 2013). Some fungi have metabolic products that are toxic to human hosts aflatoxins are produced by the development of fungi *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and *A. pseudotamarii*, when they find suitable humidity and temperature parameters (SIAP, 2023; Popescu et al 2022). The fungal contamination of chili peppers depends on factors such as humidity, temperature, drying, and processing conditions. Lack of cleanliness during harvesting or storage and air drying of chili peppers increases the risk of fungal growth and subsequent mycotoxin production (SIAP, 2023; Singh and Coty, 2019). There are four main aflatoxins: B1, B2, G1, and G2, B1 being the most important because it is considered the most potent natural carcinogen (Martínez et al 2013). When food contaminated with aflatoxin B1 is ingested, it is first absorbed in the small intestine and then transported into the blood to the liver, where it accumulates and is metabolized, forming compounds that can cause liver damage, mutations, and cancer (Costa et al 2019; Singh and Coty, 2019; Martínez et al 2013). Based on the above, the main objective of this work was to determine the presence of *Aspergillus flavus* Link and *A. parasiticus* Speare in samples of dried chili peppers marketed in Oaxaca, Mexico.

Materials and methods

Sampling and representativeness of the sample

A tour of the city's markets was conducted: Benito Juárez, Sánchez Pascuas, La Merced, Las Flores, IV Centenario, Santa Rosa, and Central de Abastos, counting 33 seed and grain establishments. They were assigned a consecutive number, and a simple random sampling was performed.

1 kg of each sample grown between November 2023 and February 2024 was acquired. The samples were five of chile de árbol, five of guajillo, and four of ancho, for fourteen samples of first-quality chile. The fraction intended for microbiological analysis was representative of the entire sample.

Moisture determination

The moisture content of the samples was determined in triplicate by oven drying (FISHER, Junior model) according to the Mexican standard (NMX-F-083-1986) and using equation 1:

$$(\% \text{moisture}) = \frac{P - P_1}{P_2} \quad (\text{Equation 1})$$

Where P = Weight of the container with wet sample in grams, P₁ = Weight of container with dry sample and P₂ = Sample weight in grams.

Total count of fungi and yeasts

Stock solution and dilutions. 10 g of crushed sample and 90 ml of isotonic solution were homogenized in a mixing beaker (0.9% NaCl). Using a sterile pipette, 1 ml of the stock solution was taken and added to a new tube with 9 ml of diluent to obtain a 10⁻¹ dilution, repeating the procedure until a 10⁻⁴ dilution was obtained.

Plate seeding. The analysis was performed in triplicate according to the Mexican standard (NOM-111-SSA1-1994), a widely recognized guideline in the field. This was done by PDA pour plate inoculation. Plates were then incubated at 25 °C in an INDELAB model IDL-CD-80 incubator. During the first 72 hours, colony forming units (CFU) were counted every 24 hours, reporting plates with 10 to 150 colonies, and expressing the results as colony forming units (CFU) per gram of agar.

Isolation and colony selection

All different morphological types of fungal colonies that appeared on the plates were isolated. Isolation was carried out by direct reseeding with a culture loop on plates with PDA medium.

Morphological identification of fungi

Identification was performed by studying the morphology by macro- and microscopy. For macroscopic identification, the colonies were reseeded by puncture on PDA medium plates and incubated at 37 °C for 7 days observing the shape, surface texture, and color of the colonies. Microscopy was performed by reseeding using the technique of Ridell (1950). The microcultures were grown on PDA medium and incubated for 48 hours at 37 °C. Microscopic observations were made and the keys of Klich (1988) were used to identify the genus and, when possible, the species, observing macroscopic characteristics such as shape, surface texture, and color, as well as microscopic characteristics of their fruiting bodies.

Presumptive determination of aflatoxin production capability

To determine the aflatoxin production capacity of the *Aspergillus flavus* and *A. parasiticus* strains isolated and identified from dried chili peppers, the technique reported by Davis (1987) was used (Davis et al 1987). Colonies were spot inoculated onto coco agar medium and incubated at 28 °C for 72 hours. They were observed with a 360-

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nm UV light lamp to identify fluorescence on the back and front of the culture medium (Bogantes et al 2004). Fungi fluoresced were aflatoxin producers.

Results

Moisture quantification and determination of fungi and yeasts

The data for fungi count, yeast, and moisture percentage are shown in Table 1. Mexican Standard NMX-FF-107/1-SCF1-2006 for food products, whole dried chiles (guajillo, ancho, mulato, de árbol, puya, and pasilla) establishes that dried chiles must be free from fungi and yeasts for marketing. All samples showed the presence of fungi and yeasts. Samples 29 and 15 of the varieties chile ancho and chile de árbol, respectively, had 100 CFU/g, being both samples the lowest counts obtained. On the other hand, in samples 5 (wide) and 32 (tree), *Aspergillus parasiticus* and *A. flavus* strains were identified, respectively (Table 2). Sample 22 of the guajillo chile variety was the one with the highest count of molds and yeasts with 6700 CFU/g. The Mexican standard NMX-FF-107/1-SCF1-2006 establishes a maximum moisture content of 12.5% for ancho chile, 9% for chile de árbol, and 13.5% for chile guajillo. All ancho chile and chile de árbol samples exceeded the percentage of moisture established by the Mexican standard with 23% and 3%, respectively. Of the guajillo chile bell pepper samples, only one met the moisture percentage established by the Mexican standard with 23% and 3%, respectively.

Morphological identification of fungi

Morphological identification of fungi isolated from dried chili peppers was performed using the methodology previously described (Riddell, 1950; Klich and Pit, 1988). Table 2 shows the species isolated from each of the samples. Six genera *Aspergillus*, *Cercospora*, *Fusarium*, *Penicillium*, *Rhizopus* and *Cladosporium* and 12 species were identified. *Aspergillus parasiticus* and *A. flavus* were identified in samples 5 and 32, respectively (Figure 1). *Aspergillus flavus* presented punctate colonies of flat elevation, with

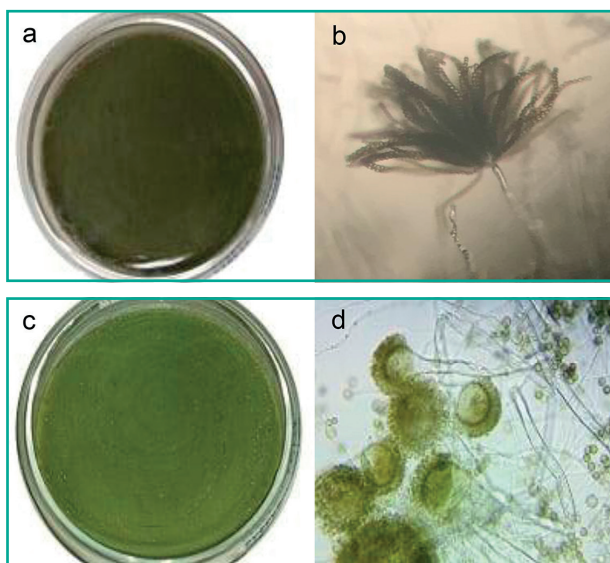


FIGURE 1: *Aspergillus flavus*: a) in PDA medium of 7 days of growth at 28 2 °C and b) microscopy at 100x. *A. parasiticus*: c) in PDA medium of 7 days of growth at 28 2 °C and d) microscopy at 100x.

TABLE 1: Fungal and yeast counts in dried chili peppers and percent moisture content.

Market	Sample	Variety of chili	CFU in PDA/g sample incubated at 25 °C for 48 hours	% moisture content in dried chili samples
La Merced	1	De árbol	3000	12.35
Benito Juárez	4	Guajillo	3700	12.52
	5	Ancho	4200	21.31
	8	De árbol	400	14.23
	9	Guajillo	5700	18.67
Sánchez Pascuas	10	De árbol	300	15.75
	15	De árbol	100	15.62
	18	Guajillo	4800	18.54
Central de abastos	21	Ancho	2000	24.99
	22	Guajillo	6700	27.81
Santa Rosa	29	Ancho	100	20.13
Las Flores	31	Guajillo	900	16.76
IV Centenario	32	De árbol	5900	12.48

TABLE 2: Fungal genera isolated and identified from dried chili peppers.

Sample	Variety of chili	Isolated genera
1	de árbol	<i>Aspergillus</i> sp <i>Aspergillus versicolor</i> <i>Aspergillus venenatus</i> <i>Aspergillus niger</i> <i>Cercospora</i>
4	guajillo	<i>Fusarium</i> <i>Oxysporum</i>
5	ancho	<i>Aspergillus parasiticus</i>
8	de árbol	<i>Rhizopus</i>
9	guajillo	<i>Fusarium solani</i>
10	de árbol	<i>Aspergillus niger</i>
15	de árbol	<i>Rhizopus</i>
18	guajillo	<i>Rhizopus</i>
21	ancho	<i>Penicillium</i> <i>Cladosporium</i>
22	guajillo	<i>Rhizopus</i>
25	ancho	<i>Penicillium</i>
29	ancho	<i>Rhizopus</i>
31	guajillo	<i>Rhizopus</i>
32	de árbol	<i>Aspergillus flavus</i>

frayed edges, powdery to wooly texture, and abundant growth. Microscopically, it presented a uniseriate and radial aspergillary head, chains of conidia supported by stipes, without metulae, globose vesicle, and covered three quarters or completely by phialides (Figures 1a, 1b). *Aspergillus parasiticus* presented colonies of green color and circular shape, flat elevation, with granular to velvety texture. Microscopically, its stipe is slightly rough, hyaline; the vesicle is globose, uniseriate, with no metulae; the phialides covered one third or the upper half of the vesicle (Figures 1c, 1d). It was not considered necessary to describe the genus of the yeasts in the chili pepper samples since they are not involved in processes that affect the eaters' health, such as the mycotoxins mentioned above.

Presumptive determination of aflatoxin production of the strains of *Aspergillus* spp.

The ability of isolated strains of *Aspergillus flavus* and *A. parasiticus* to produce aflatoxins in coco agar medium

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was evaluated. When exposed to 360-nm UV light, both strains fluoresce in the medium and are therefore considered to produce aflatoxins (Figure 2).

Discussion

The following microorganisms were found in the samples analyzed: *Fusarium oxysporum* is a fungus that plays a significant role in the storage of dried chili peppers. It is one of the main pathogens associated with wilt and rot of these products. This fungus can affect both plants in the field and stored fruits, causing damage that compromises the quality and viability of chili peppers. Our studies, conducted across diverse regions, have unearthed a disquieting truth. *F. oxysporum* is a pervasive presence in a substantial percentage of chili bell pepper samples. This fungus, with its telltale symptoms of chlorosis, flaccidity, and root and neck rot, poses a significant threat to the quality and yield of the crop, and in severe cases, can lead to complete crop failure (Cai et al 2022). The genus *Penicillium* can be frequently present in stored dried chili peppers. Some *Penicillium* species have the potential to cause pathological spoilage in dried produce (Martín et al 2022). *Rhizopus* is another genus that can occur in dried chili peppers with longer storage times. Certain *Rhizopus* species can reach 100% infestation levels in dried chili peppers without germination power (Liu et al 2024).

The presence of aflatoxigenic strains in food represents a major health problem, since their toxins can cause diseases that cause health damage or even death in people or animals that consume contaminated food. These are very common fungal contaminants in a wide range of agricultural products, deteriorating them and making them a threat to consumers (Akhund et al., 2017). Fungal contamination can occur at any stage of food production from field to storage as soon as the right conditions are obtained for its development (Martínez et al 2013; Iqbal et al 2010). Drought conditions, together with a relative humidity of 85% and a temperature of 27 to 38 °C, are the best option for the growth of fungi of the genus *Aspergillus* and their consequent production of aflatoxins, conditions that are readily available in Oaxaca. Different authors have reported contamination of chili peppers from markets by aflatoxins, mainly produced by *A. flavus* as they go through different processes, such as drying, transport, or storage, where chili peppers are exposed to airborne *Aspergillus conidia* at appropriate temperature and humidity (Rosas-Contreras et al 2015; Akhund et al 2017). The treatment that chili peppers receive for marketing and storage is also of utmost importance, since chili peppers with high moisture content are a suitable substrate for the development of aflatoxigenic strains and consequent contamination with aflatoxins, as described by Sahar (2015) who reported that of the chili samples they analyzed (Sahar et al 2015), those with the highest percentage of moisture had the highest aflatoxin contamination, highlighting the importance of using dried chili peppers with very low moisture content to minimize the health risks associated with aflatoxin contamination. The humidity values of the samples analyzed in this study exceed those allowed by the Mexican standard, which is an important factor for possible contamination by *Aspergillus*. Other studies have been carried out to determine the fungal biota that contaminate food, as well as the metabolites that are present in them. Rosas-Contreras (2015)

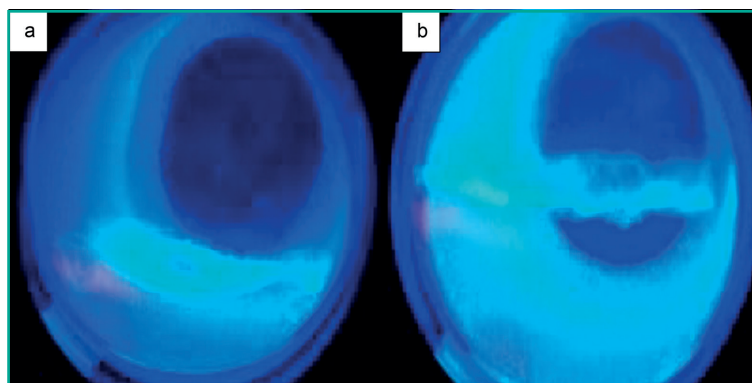


FIGURE 2: Identification of aflatoxin production capacity in 4-day incubation coco agar medium of the strains: a) *Aspergillus parasiticus* and b) *Aspergillus flavus*.

identified and quantified aflatoxins in 64 samples of three varieties of ancho, guajillo, and piquin chili peppers most marketed in Mexico City (Rosas-Contreras et al 2015). All samples were contaminated with some type of aflatoxin. Chili peppers marketed in Mexico City were exposed to the presence of aflatoxigenic strains, as were the chili peppers analyzed in this study, because strains of *Aspergillus flavus* and *A. parasiticus* producing aflatoxins were identified. Iqbal (2010) analyzed chili peppers marketed in Pakistan from rural, semi-rural and urban areas (Iqbal et al 2010). The levels found for the samples exceeded the maximum limit for the European Union, with chili peppers from rural areas being the most contaminated, suggesting that the origin of the chili peppers is closely related to their quality, since marketing conditions vary depending on the point of sale. Likewise, the strategy of eliminating *Aspergillus* strains from chili peppers may be insufficient as a control measure, since it does not guarantee the elimination of aflatoxins, which are very difficult to eradicate (Russell and Paterson, 2006). Set (2014) found that out of 120 samples of red chili peppers marketed in Turkey, all had aflatoxin contamination, concluding that there is a need to evaluate the quality of marketed chili peppers and to prohibit the distribution and sale of those with mycotoxin contamination (Set and Erkmén, 2014). Wikandari (2020) found that 80 % of the total samples analyzed presented infection by *Aspergillus* spp. of which 50 % were identified as *Aspergillus flavus* and *A. parasiticus*; In addition, they found the presence of aflatoxins B1 and B2 produced by these fungi (Wikandari et al 2020). Aflatoxins are mycotoxins that are harmful to the health of those who consume them. Chigoziri (2013) isolated a total of 20 genera and 36 species of fungi from 800 bell pepper seeds produced in areas of Nigeria (Chigoziri and Ekefan 2013). *Colletotrichum capsici*, *Aspergillus niger* and *A. flavus* were the most frequently isolated fungi, with *A. flavus* being a very important fungus, since under the right conditions it can produce aflatoxins. Singh (2018) analyzed 55 samples of chili peppers marketed in Nigeria and 169 in the United States, detecting the presence of *Aspergillus flavus* in 84% of the samples and *A. parasiticus* in 0.4% (Singh et al 2018). In assessing whether they were aflatoxin producers, they found that some strains produced only aflatoxin B, while others could produce aflatoxin B and G. In analyses of other foods, such as that performed by Arrus (2005) on Brazilian walnuts, they identified the presence of *Aspergillus flavus* and *A. parasiticus*, and when testing for aflatoxin production of both strains, they found high levels of pro-

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duction of the toxin, in addition to contamination in samples of walnuts in storage (Arrus et al 2005). Ochoa (2007) determined the fungal biota in oranges, from which they isolated and identified three genera of fungi, *Aspergillus*, *Fusarium* and *Penicillium*, and five species, including *Aspergillus flavus*, which causes fruit rot (Ochoa et al 2007). Luna (2010) carried out the isolation of *Aspergillus niger* strains in samples of parchment coffee in storage in Mexico, where all the isolated strains confirmed to be producers of ochratoxins when they find the adequate parameters of temperature and humidity for the development of the fungus (Luna and Trigos 2010). The contamination of food by fungi can be mainly due to the handling, transfer, or exposure of food for storage, sale, or consumption, since there are opportunistic fungi that can contaminate them and under appropriate conditions produce toxins that cause damage to health and economic and production losses. Fungi can be eliminated by thermal or cooking processes, however, once they produce mycotoxins, it is very difficult to eliminate them and they can reach the final product (Asai et al 2012). It is important to know the quality of food, as well as to work on strategies to improve its conditions during the production and commercialization processes.

Conclusions

Aflatoxins are carcinogenic substances produced mainly by *Aspergillus flavus* and *A. parasiticus*. In this work, six genera of fungi were identified in the fourteen samples of dried chili peppers analyzed, these were *Aspergillus*, *Cercospora*, *Fusarium*, *Rhizopus*, *Penicillium*, *Cladosporium*, in the varieties of chili de árbol, guajillo and ancho. The strains of *Aspergillus flavus* and *A. parasiticus* were identified in a sample of chile de árbol and a sample of chile ancho, respectively, and when evaluating their capacity to produce aflatoxins, both strains were positive, which would represent a high risk since they can develop, making them a latent risk for the population. It is important to carry out constant monitoring of the quality of the chili peppers marketed at the different points of sale, since contamination by fungi can lead to serious health problems for people who meet the product, whether they are producers or consumers, as well as representing significant economic losses for the productive sector in this area.

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None.

Ethics statement

Not applicable.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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