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Determination of Mycotoxigenic Fungi and Total Aflatoxins in Stored Corn from Sites of Puebla and Tlaxcala, Mexico

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ABSTRACT

This paper aimed to evaluate the contamination with mycotoxigenic fungi and total aflatoxins in stored corn from different sites in Puebla and Tlaxcala, Mexico. Methodology. The study was conducted at two sites in Puebla (San Salvador El Seco and Junta Auxiliar La Resurrección) and two sites in Tlaxcala (Tlaltepango and Nativitas). A total of 80 samples of stored corn were collected. Identification of *Aspergillus flavus* was performed by microculture techniques and specific taxonomic keys (macromorphological and micromorphological). Then, samples of contaminated corn were selected, and aflatoxin production was confirmed using a direct solid-phase ELISA kit. A total of 25 *A. flavus* strains were identified. Other possible mycotoxin-producing fungi were *Penicillium* (n=52) and *Fusarium* (n=19). Regarding total aflatoxin contamination, all samples were contaminated within a range of 1.589 to 11.854 µg/kg, and the average concentration was 6.3 µg/kg corn. Implications. The detection of mycotoxigenic fungi in the samples tested and of aflatoxins in corn highlights the importance of monitoring these fungi. Since food safety is at risk, it shows the need for methods to control these fungi and their metabolites.

INTRODUCTION

Mycotoxins are toxic secondary metabolites that contaminate a variety of foods. Their consumption can be harmful to human and livestock health, as these metabolites can cause teratogenesis, immunosuppression, and cancer. These are substances produced mainly by strains of the genera *Penicillium, Fusarium,* and *Aspergillus*. The most studied mycotoxins are aflatoxins and the most toxic naturally produced mycotoxins. There are about 20 types of aflatoxins, with aflatoxin B1 being the most toxic and classified as carcinogenic by the International Agency for Research on Cancer (WHO & IARC 1993). Numerous studies have shown that aflatoxins can be carcinogenic, teratogenic, mutagenic, and hepatotoxic in both animals and humans (Díaz de León-Martínez et al. 2020).

Aflatoxins are produced mainly by *Aspergillus flavus*. This and other fungi colonize numerous cereals, including corn. Colonization and degradation of plant tissues occurs through enzymatic degradation of starch, proteins, and lipids (Lu et al. 2022). This occurs in response to stimuli and threats from the environment. It is known that most mycotoxins, including aflatoxins, can be taken up from the root in plants such as corn and then translocated to organs above the soil (Righetti et al. 2021). This colonization and production of mycotoxins is influenced by factors such as temperature, moisture, and nutrient content of corn grain. Therefore, grain storage conditions are critical to avoid this contamination by mycotoxic fungi and their metabolites. Corn is a crop of global importance and ranks third in the world, followed by wheat and rice, due to its high consumption (De Girolamo et al. 2016).

In Mexico, corn is a staple food and the main crop for domestic use, especially in rural areas. Climate change has created favorable conditions for the spread of fungi and their metabolites in places where this was not previously the case (Saez-Gomez et al. 2022). Contamination of these and other crops threatens food security. Therefore, monitoring is important to prevent the spread of these fungi and their metabolites. However, there is no uniform regulation worldwide for the permissible limits of these metabolites in cereals and their finished products. In Mexico, there is a National Standard (NOM-188-SSA1-2002) that establishes permissible limits for total aflatoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2) in cereals for human and animal consumption. These limits state that the total content of aflatoxins in cereals must not exceed 20 µg/kg. If the concentration is between 21 and 300 µg/kg, the grain should be destined for animal feed. However, the European Union has more specific standards for aflatoxin B1, as 2 µg/kg-1 should not be exceeded in food for human consumption (Reinhold & Reinhardt 2011).

Techniques for monitoring aflatoxins in food include rigorous methods such as chromatographic techniques, e.g., thin-layer chromatography and HPLC. However, these methods are expensive and require extensive sample preparation. For this reason, the use of ELISA kits serves as a replacement technique because these tests are rapid, sensitive, specific, and less expensive (Tarannum et al. 2020). In a study conducted by Cabrera-Meraz et al. (2021), total aflatoxin contamination was quantified in samples of corn kernels, dough, and tortillas, with values ranging from 0.82 to 28.04, 0.66 to 14.36, and 0.63 to 12.04 $mg.kg^{-1}$, respectively, in 75% of the samples. Similarly, Jayaratne et al. (2020) reported aflatoxin B1 in corn kernels with concentrations of 60-70 ppb, while these concentrations in agricultural soils were 350-400 ppb. In another study by Rojas Jaimes et al. (2020), contamination by total aflatoxins was found in peanuts with a concentration of 149.7 ppb.

Monitoring of these metabolites is important from an economic point of view because of financial losses when crop yields decrease or because of the costs of prevention or decontamination. This work aimed to evaluate contamination by total aflatoxins (aflatoxin B1, aflatoxin B2, aflatoxin B3, and aflatoxin B4) in corn stored in the regions of Puebla and Tlaxcala, Mexico.

MATERIALS AND METHODS

Study Area and Sample Collection

The study was conducted at different sites: San Salvador El Seco (1909′50.50″N, 97037′48.50″W) and Junta Auxiliar La Resurrección (1906′50.30″N, 9808′10.50″W) in the State of Puebla (SSS and JAR, respectively). In Tlaltepango (1907′15.79″N, 9809′18.51″W) and Nativitas (19014′3.50″N, 98018′41.50″W) in Tlaxcala State (TLA or

Fig. 1: Test sites: Puebla (San Salvador El Seco and Junta Auxiliar la Resurrección) and Tlaxcala (Tlaltepango and Nativitas). Mapa Digital de México V6.3.0, 2023.

NAT). A total of 80 samples of corn were collected in January (n=40) and August (n=40) 2017. In the state of Puebla, 40 samples were collected from SSS as well as JAR, while in the state of Tlaxcala, 20 samples were collected from TLA and 20 samples from NAT (Fig. 1). Corn was collected from different storage locations (10 locations). In some locations, the corn was stored unpackaged, and only two locations used cloth bags (50 kg). Using a sterile plastic hand scoop. Approximately 100 g of samples were placed in Ziploc bags and transported to the laboratory, where they were stored at 4ºC until use.

Isolation and Phenotypic Identification of Presumptive Isolates of *A. flavus*

Samples were pounded in a mortar until a fine powder was obtained. From these samples, 1:10, 1:100, 1:1000, and 1:10,000 dilutions were prepared. Subsequently, 50 µL of the last two dilutions of each sample were inoculated into Sabouraud dextrose agar (SDA, Sigma-Aldrich, Mexico) and incubated at 28 °C for 7 days. Presumptive colonies of *Aspergillus* section *Flav*i were seeded in tubes containing SDA to obtain axenic cultures. Subsequently, the microculture technique was applied to perform macromorphological and micromorphological identification using specific taxonomic keys (Rippon & Castañeda 1992, Bonifaz 2015). The putative isolates of *A. flavus* and *A. parasiticus* were reseeded in an AFPA differential medium (*Aspergillus flavus parasiticus* agar, Sigma-Aldrich, Mexico). To confirm their identification by the production of orange-yellow pigment on the back of the colony.

Quantification of Total Aflatoxins

A direct competitive solid-phase ELISA kit (Astori S.N.C., Poncarale, Italy) was used to determine total aflatoxin contamination in corn samples. An extraction solution (70% methanol, Sigma-Aldrich, Mexico) was prepared from each sample. Once the sample was prepared, it was ground (Hamilton Beach Model 80335R, USA) until it had the particle size of fine instant coffee. Then, 1g of the sample was weighed, and 5 mL of the extraction solvent was added. The whole was mixed with stirring for about 2 min, the mixture was allowed to stand until it precipitated, and then it was filtered through Whatman #1 filter paper. The filtrate obtained was analyzed as mentioned below.

Analysis of the filtrate obtained from the sample: According to the sample analyzed, 200 µL of the aflatoxin-HRP conjugate was added to each dilution well, 100 µL of each standard and sample was added to the corresponding dilution well containing the conjugate, 100 µL of the contents of each dilution well was pipetted three times and transferred to a corresponding antibody-coated microtiter well, incubated at room temperature for 15 min, and then the contents of the microtiter wells were transferred to a waste cell. The microtiter wells were washed 5 times by filling each well with PBS-Tween wash buffer (Sigma-Aldrich, Mexico). Then, the remaining buffer was removed, 100 µL of substrate reagent was added to each microtiter well and incubated at room temperature for 5 min, followed by 100 µL of stop solution. Finally, the optical density (OD) of each microplate was read with a microplate reader (Accuris MR9600, USA) using a 450 nm filter. Concentrations were calculated by extrapolating the OD with the respective calibration curve generated from the following concentrations: 1.0, 2.5, 5.0, and 10.0 $\mu g.kg^{-1}$.

RESULTS AND DISCUSSION

The states of Puebla and Tlaxcala are located in the center of Mexico. Their climate is temperate and semi-humid. The average daily temperature is 25°C, but there is considerable seasonal variation. The rainy season lasts about 7 months, and humidity is very high during this period (Briones-Reyes et al. 2007). Corn is the most important agricultural product in both states. However, corn is also the most consumed product for humans. Considering the climatic conditions, with the prevailing hot and semi-humid conditions for considerable periods of time, there is a high probability of infestation with corn fungi during agricultural processes, grain storage, and processing (Domínguez-Hernández et al. 2022). In addition, Rodríguez-Ramírez et al. (2021) reported temperature and precipitation variability in this geographic zone during 2015-2017. They noted an increase in insect populations due to higher temperatures and an increase in fungi due to humidity.

Based on micromorphological and macromorphological characteristics (Fig. 2) and using specific taxonomic keys, *A. flavus* species were identified in native corn kernels stored outdoors at the four test sites. Colonies of *A. flavus* were apartment, greenish-yellowish, with white margins and spherical spores. In addition, strains positive for the morphological characteristics of *A. flavus* were seeded in the AFPA differential medium to be sure that they had been treated with this species since strains of this fungus produce a yellow-orange pigmentation on the back of the colony (Fig. 3). A total of 25 strains of *A. flavus* were isolated; Fig. 4 shows the number of isolates obtained at each test site. Similarly, strains of *Fusarium* and *Penicillium* were also identified. Fig. 5 shows that the greatest diversity of identified genera was obtained in JAR, followed by TLA, NAT, and SSS. However, only two genera were found in the latter: 3 isolates of *Penicillium* and 1 of *Fusarium*.

For the count of total aflatoxins in the 80 corn samples, a calibration curve was generated by linear regression with

Table 1: Concentration of total aflatoxins in stored corn collected from different sites in Puebla and Tlaxcala, Mexico.

Strains	Absorbance	Concentration[µg.kg^{-1}]	Strains	Absorbance	Concentration[µg.kg^{-1}]
2S-M5	0.765	5.637	2N-M4	0.645	8.239
2J-M5	0.985	3.516	2T-M2	0.495	6.215
2N-M5	0.86	4.721	1S-M9	0.705	*10.552
1T-M10	0.76	5.685	2J-M1	0.255	1.589
2T-M5	0.915	4.191	2N-M1	1.185	4.191
2S-M1	0.388	**9.27	1J-M6	0.915	8.769
2S-M4	0.975	3.613	2T-M1	0.44	5.203
2J-M4	0.423	8.933	1J-M10	0.81	**9.328
1N-M10	0.525	7.95	1N-M9	0.382	6.649
1T-M9	0.72	6.07	1J-M8	0.66	**9.637
2T-M4	0.408	**9.078	1T-M7	0.35	6.553
1S-M10	0.12	*11.854	1S-M8	0.67	8.384
2S-M3	0.935	3.998	1J-M9	0.48	6.89
2J-M3	1.09	2.504	1N-M8	0.635	7.227
2N-M3	0.975	3.613	1S-M7	0.6	**9.107
1N-M6	0.71	6.167	1T-M6	0.405	5.203
2T-M3	1.08	2.601	1S-M6	0.81	6.408
2S-M2	0.67	6.553	1J-M7	0.685	7.468
2N-M2	1.04	2.986	1N-M7	0.575	6.167
2J-M2	0.71	6.793	1T-M8	1.13	2.119

Total aflatoxin concentrations: * greater than 10 µg.kg^{-1} of maize; ** close to 10 µg.kg^{-1} of maize

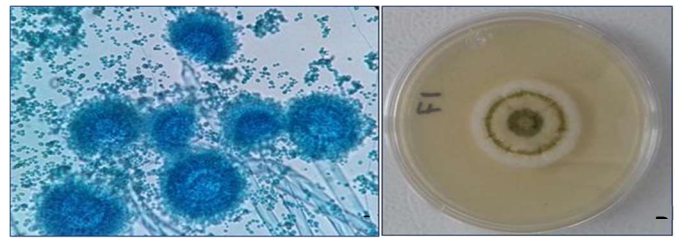


Fig. 2: Micromorphological (A) and macromorphological (B) characteristics of *Aspergillus flavus* isolated in stored corn collected from different sites in Puebla and Tlaxcala, Mexico.

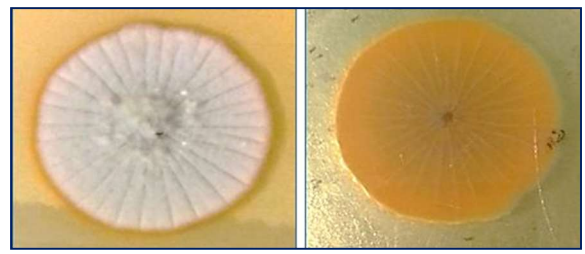


Fig. 3: Front and back of the *Aspergillus flavus* colony in AFPA differential medium isolated in stored corn collected from different sites in Puebla and Tlaxcala, Mexico.

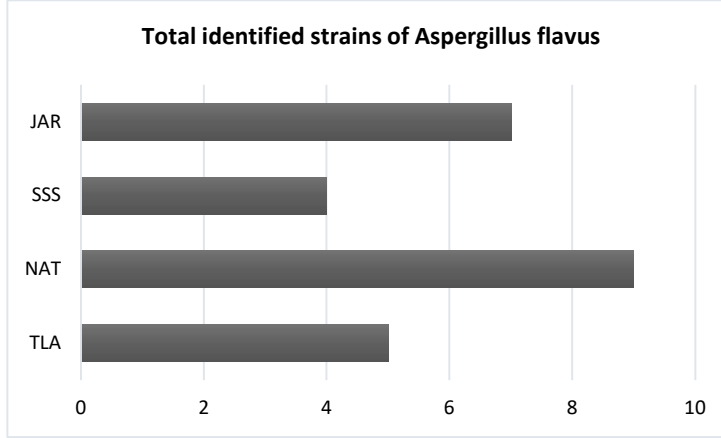
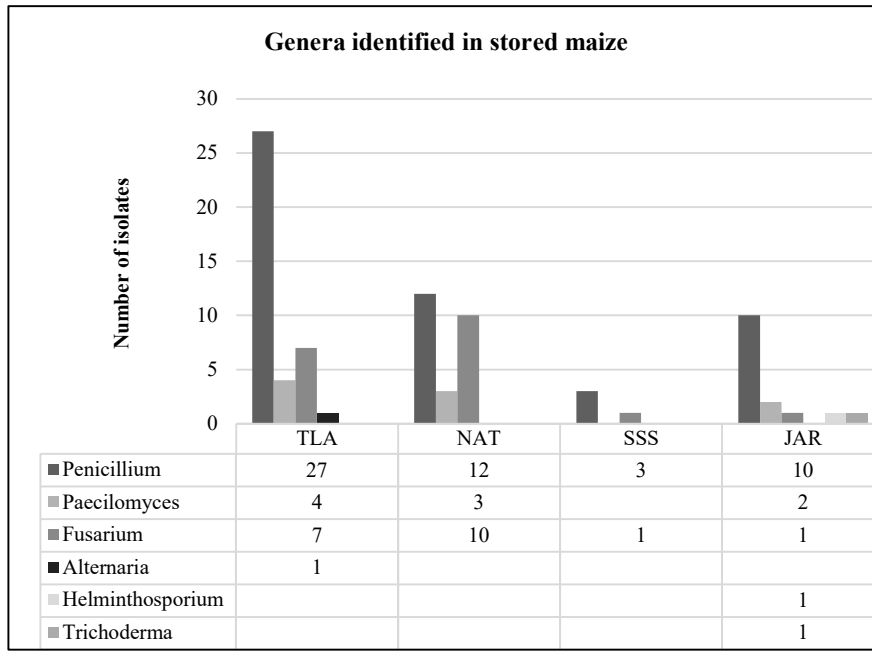


Fig. 4: Number of *Aspergillus flavus* isolated in stored corn collected from different sites in Puebla and Tlaxcala, Mexico.



	TLA	NAT	SSS	JAR
Penicillium	27	12	3	10
Paecilomyces	4	3		2
Fusarium	7	10	1	1
Alternaria	1			
Helminthosporium				1
Trichoderma				1

Fig. 5: Genera identified in isolated stored corn collected from different sites in Puebla and Tlaxcala, Mexico.

the total aflatoxin standards at different concentrations. The results of the analysis of the corn samples are shown in Table 1. *A. flavus* is a potentially aflatoxigenic fungus. Inadequate storage conditions, controlling temperature and humidity, could be a critical factor in the formation of aflatoxins, toxic secondary metabolites commonly associated with liver cancer (Bbosa et al. 2013). The characterization of macro- and micromorphology is consistent with that of Seerat et al. (2022). In addition, fewer isolates of this aflatoxigenic species were obtained in SSS, possibly due to the temperate and semi-arid climate there. Although *Aspergillus* is a cosmopolitan fungus that occurs in different climates, according to Bonifaz (2015), the climate that favors its spread is a humid climate, which is also present in the other sampled sites. Based on these results, climate is a factor contributing to the spread of aflatoxigenic fungi and, in this study, to the production of *A. flavus*, the only aflatoxigenic species isolated in the sampled sites.

In addition, contamination with this fungus may begin during the field phase and increase during the storage phase. This is mainly due to poor grain storage practices, e.g., if the temperature is not controlled, if the contaminated grains are not removed, they may contaminate other grains, and if there are insect or mite populations, they may damage the

grains and make them more susceptible to contamination. These factors may have influenced the fact that the corn was contaminated with *A. flavus* because, at the sampling sites, the locations designated for corn storage were outdoors.

In a study by Seerat et al. (2022), contamination with *A. flavus* was also found in corn samples. 212 isolates were recovered from 80 analyzed corn seed samples. On the other hand, Wokorach et al. (2022) reported that *A. flavu*s was present in 63% of staple food samples. Similarly, Okoth et al. (2012) reported isolates of *A. flavu*s planted in AFPA with a yellow-orange coloration on the back of the colony. Contamination by this potentially aflatoxigenic fungus in these substrates (agricultural soils and corn) is an ongoing threat to human and animal health at the sites where sampling was conducted. This is because the crops are destined for consumption by the farmers' families or sold in the region. Therefore, the presence of these high aflatoxigenic species affects access to nutritious and culturally appropriate foods derived from these crops.

Other filamentous fungi identified and of economic importance were *Penicillium*, *Fusarium*, and *Alternaria*, some species of which are also potentially aflatoxigenic. Based on the number of strains found, we can assume that only the isolates of *Penicillium*, *Paecilomyces*, and *Fusarium* are important. In a study by Penagos-Tabares et al. (2022), the presence of *Penicillium roqueforti, Saccharomyces* spp., *Geotrichum candidum, Aspergillus fumigatus, Monascus ruber, Mucor circinelloides, Fusarium* spp., and *Paecilomyces niveus* was detected in corn silage. As you can see, the genera *Penicillium, Aspergillus,* and *Fusarium* are consistent with those reported in this study.

As for the quantification of total aflatoxins, contamination with total aflatoxins (AFB1, AFB2, AFB3, AFB4) was detected in all samples. The average concentration of aflatoxins in the samples tested was 6.3 $\mu g.kg^{-1}$ of maize. In 2 of these samples, the concentration was above 10 µg/kg, and in 5, it was close to this concentration. Although none of the samples exceeded the value of NOM -188-SSA1-2002, these concentrations are above the permissible values compared to the European standards. The fact that contamination with total aflatoxins was detected in all the samples tested (n=40) indicates that the storage conditions for this cereal are not optimal. Moreover, the presence of these metabolites in a cereal that is frequently consumed in the region where the sampling took place endangers the health of the inhabitants, as they are the main consumers.

It has also been shown that the ELISA technique contributes to the rapid identification of these metabolites. It is a cost-effective, sensitive, and reliable technique. Moreover, an analytical method with these characteristics is essential to identify agricultural products contaminated with these metabolites or other mycotoxins to prevent them from becoming final products and not being marketed.

Contamination by these metabolites in a cereal that is widely in our country, and especially in the sampled areas, poses a risk to human and animal health because of the effects these metabolites can cause since the sampled corn is marketed in the region. They also affect the nutritional and commercial value and threaten the family income of producers since agriculture is one of the main economic activities in the sampled areas.

In summary, this study shows that corn at sites in Puebla (SSS and JAR) and Tlaxcala (TLA and NAT) is susceptible to general aflatoxin contamination. *A. flavus*, a potentially aflatoxigenic strain, can also affect the quality of this grain and, at the same time, human and animal health. Therefore, it is important to adopt stricter regulations for total allowable aflatoxin levels in grains such as corn since they are so important for consumption. Finally, the ELISA technique for determining total aflatoxin content in corn is a simple, rapid, and practical technique. This can contribute to sustainable agriculture.

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REFERENCES

Bbosa, G.S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W.W. and Kyegombe, D.B. 2013. Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems. In Razzaghi-Abyaneh, M. (ed.) Aflatoxins-Recent Advances and Future Prospects. InTech, Lodon, UK, pp. 239-265.

Bonifaz, J. (ed.) 2015. Basic Medical Mycology. McGraw Hill, Mexico, p.725.

Briones-Reyes, D., Gómez-Martinez, L. and Cueva-Rolón, R. 2007. Zearalenone contamination in corn for human consumption in the state of Tlaxcala, Mexico. Food Chem., 100(2): 693-698.

Cabrera-Meraz, J., Maldonado, L., Bianchini, A. and Espinal, R. 2021. Incidence of aflatoxins and fumonisins in grain, masa, and maize tortillas in four municipalities in the department of Lempira, Honduras. Heliyon, 7(12): e08506.

De Girolamo, A., Lattanzio, V.M.T., Schena, R., Visconti, A. and Pascale, M. 2016. Effect of alkaline cooking of maize on the content of fumonisins B1 and B2 and their hydrolyzed forms. Food Chem., 192(2): 1083-1089.

Díaz De León-Martínez, L., Rodríguez-Aguilar, M., Wong-Arce, A., Díaz-Barriga, F., Bañuelos-Hernández, B., Rosales-Mendoza, S. and Flores-Ramírez, R. 2020. Evaluation of acute and chronic exposure to aflatoxin B1 in indigenous women of the Huasteca Potosina, Mexico. Env. Sci. Poll. Res., 27(5): 30583-30591.

Domínguez-Hernández, E., Gaytán-Martínez, M., Gutiérrez-Uribe, J.A.

and Domínguez-Hernández, M.E. 2022. The nutraceutical value of maize (*Zea mays* L.) landraces and the determinants of its variability: A review. J. Cereal Sci., 103: 103399.

Jayaratne, W.M.S.C., Abeyratne, A.H.M.A.K., De Zoysa, H.K.S., Dissanayake, D.M.R.B. N., Bamunuarachchige, T.C., Waisundara, V.Y. and Chang, S. 2020. Detection and quantification of Aflatoxin B1 in maize and maize-grown soils in the district of Anuradhapura, Sri Lanka. Heliyon, 6(10): e05319.

Lu, Y.N., Shan, Y., Huang, H., Zhu, L., Li, B., Wang, S. and Liu, F. 2022. Quantum dot microsphere-based immunochromatography test strips enabled sensitive and quantitative on-site detections for multiple mycotoxins in grains. Food Chem., 376(5), 131868.

Mexican Ministry of Health. 2002. Products and Services. Aflatoxin Control in Cereals for Human and Animal Consumption. Sanitary Specifications. SEGOB, Mexico

Okoth, S., Nyongesa, B., Ayugi, V., Kang'ethe, E., Korhonen, H. and Joutsjoki, V. 2012. The toxigenic potential of Aspergillus species occurring on maize kernels from two agroecological zones in Kenya. Toxins, 4(11): 991-1007.

Penagos-Tabares, F., Khiaosa-ard, R., Schmidt, M., Pacífico, C., Faas, J., Jenkins, T., Nagl, V., Sulyok, M., Labuda, R. and Zebeli, Q. 2022. Fungal species and mycotoxins in moldy spots of grass and maize silages in Austria. Mycotox. Res., 38(2): 117-136.

Reinhold, L. and Reinhardt, K. 2011. Mycotoxins in foods in Lower Saxony (Germany): results of official control analyses performed in 2009. Mycotox. Res, 27(1), 137-143.

Righetti, L., Bhandari, D.R., Rolli, E., Tortorella, S., Bruni, R., Dall'asta, C. and Spengler, B. 2021. Unveiling the spatial distribution of aflatoxin B1 and plant defense metabolites in maize using AP-SMALDI mass spectrometry imaging. Plant J., 106(1): 185-199.

Rippon, J. and Castañeda, L. (eds.) 1992. Medical Mycology: Pathogenic Fungi and Actinomycetes. McGraw-Hill, México, p.321.

Rodríguez-Ramírez, E.C., Williams-Linera, G., Díaz-Ávalos, C. and Luna-Vega, I. 2021. Masting effect on canopy greenness and climate response on seed production of *Fagus grandifolia* subsp. Mexicana across the Sierra Madre Oriental, Mexico. Clim. Change Ecol., 2: 10035.

Rojas Jaimes, J., Chacon-Cruzado, M., Diaz-Tello, A. and Castañeda-Pelaez, L. 2020. Quantification of carcinogenic aflatoxins in unprocessed foods and their implication for consumption in Lima, Peru. Nutr. Hosp., 38(1): 146-151.

Saez-Gomes, K.C., Castañeda-Roldán, E.I., Avila-Sosa, R. and Munguía-Pérez, R. 2022. Mycotoxins and Climate Change. In: Frías-De-León, M.G., Mendoza, C.B., Montes, M.D.R.R. and Escalante, E.D. (eds), The Impact of Climate Change on Fungal Diseases: Fungal Biology. Springer, Switzerland, pp. 239-256.

Seerat, W., Akram, A., Qureshi, R., Yaseen, G., Mukhtar, T. and Hanif, N.Q. 2022. Light and scanning electron microscopic characterization of aflatoxins producing *Aspergillus flavus* in the maize crop. Micro. Res. Tech., 85(8): 2894-2903.

Tarannum, N., Nipa, M.N., Das, S. and Parveen, S. 2020. Aflatoxin M1 detection by ELISA in raw and processed milk in Bangladesh. Tox. Rep., 7(5): 1339-1343.

Wokorach, G., Landschoot, S., Lakot, A., Karyeija, S.A., Audenaert, K., Echodu, R. and Haesaert, G. 2022. Characterization of Ugandan endemic Aspergillus species and identification of non-aflatoxigenic isolates for potential biocontrol of aflatoxins. Toxins, 14(5): 304-307.

World Health Organization and International Agency for Research on Cancer (WHO & IARC) 1993. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines, and mycotoxins.