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Mycological and Mycotoxicological Analysis of Maize Imported into Senegal

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ABSTRACT

Most of the maize used in Senegal comes from abroad. However, no contamination data is available to date for maize imported into Senegal. The objective of this study is to determine the food safety level of maize from France, Argentina and the United States. The analysis of aflatoxins in corn samples is carried out on a High-Performance Liquid Chromatograph. PDA culture medium is used for the isolation, purification and identification of potentially mycotoxinogenic molds contaminating imported corn. The results of the analyzes show the presence of aflatoxins in all the samples. In 35% of the samples, the levels of aflatoxin contamination exceed the standards set by the European regulations in force. They also reveal the presence of mycotoxinogenic molds in this corn. These are two aflatoxin-producing Aspergillus (A. flavus and A. parasiticus) and Penicillium sp. As a result, and faced with this threatening situation for public health, Senegal must take a major interest in ensuring that imported products comply with international health standards.

Keywords
Maize, Aspergillus, Aflatoxin, Import, Senegal.

Introduction
Food-borne illnesses are one of the most worrying problems worldwide. Among biological contaminants, the contamination of food products by mycotoxins has recently been recognized by the World Health Organization (WHO) as an important source of foodborne diseases [1]. The main mycotoxins that pose public health problems are aflatoxins, ochratoxins, fumonisins, zearalenone and patulin [2-4]. They are produced by molds belonging mainly to the Aspergillus, Penicillium and Fusarium types. These mycotoxinogenic fungi that contaminate peanuts are also frequent contaminants of staple cereals such as maize [5].

In Senegal, corn is the third most consumed cereal in the country after rice and millet [6]. However, maize production at the national level is very low and cannot meet the consumption needs of the populations [7]. Most of the quantities used come from abroad and are mainly imported by the poultry and livestock feed manufacturing industries [7]. According to the National Agency for Statistics and Demography, the volume of maize imports is estimated at only 396,441 tons between January and July 2019 [8]. In addition, no contamination data is available to date for maize imported into Senegal [9].

A survey of flour mills in the country reveals that no health checks are carried out on imported maize despite the risk of fungal and mycotoxic contamination. Importers rely solely on health information given by suppliers of the maize countries of origin without taking into account the sanitary conditions of storage, transport, and packaging of maize [10]. This chapter will therefore address this lack of health data on maize imported into Senegal [9].

Material and Methods
Sampling
Thirty maize samples of 1 kg each were taken from agri-food industries (GMD, FKS, SEDIMA, AVISEN, NMA) which are the main players in importing maize in Senegal. Each 1 kg sample is made up of a mixture of 4 sub-samples of 250 g of seeds taken from

various points throughout the lot. The samples packaged in sterile plastic bags were transported to the laboratory where they were subjected to microbiological and mycotoxicological analyses.

Aflatoxin Analysis

The analysis of aflatoxins in the corn samples was carried out on a High-Performance Liquid Chromatograph (HPLC) (**Shimadzu, Kyoto, Japan**) which is the reference method for the quantitative detection of aflatoxins.

Mycotoxinogenic Molds Isolation and Identification

Potato Dextrose Agar (PDA) culture medium is used for the isolation, purification and identification of molds.

Grain Surface Disinfection

The grains were placed in a sieve basket with a mesh of 05-1 mm ("mosquito net" type) and followed the following disinfection cycle:

- 10 min in a beaker containing 300 ml of a solution of calcium hypochlorite (5%) and 3 drops of triton
- 1 min in a beaker containing 300 ml of sterile water
- 1 min in a beaker containing 300 ml of sterile water

During each of the three phases, the basket is shaken vigorously in order to resuspend the grains and thus ensure more effective disinfection and rinsing.

The basket containing the grains is then drained and the grains are placed to dry on sterile filter paper (allow 15 min minimum under the laminar flow hood) at room temperature.

Molds Isolation and Purification

The disinfected seeds were placed, using sterile forceps, in Petri dishes containing the PDA medium at the rate of three seeds per dish (Figure 1). The whole was incubated at 32°C in an oven for 3 to 7 days. Then, an isolation of the strains of interest is carried out on the profusion of fungi that have developed on the PDA. A successive subculture in points by exhaustion is carried out on the strains of interest until visually pure colonies are obtained.

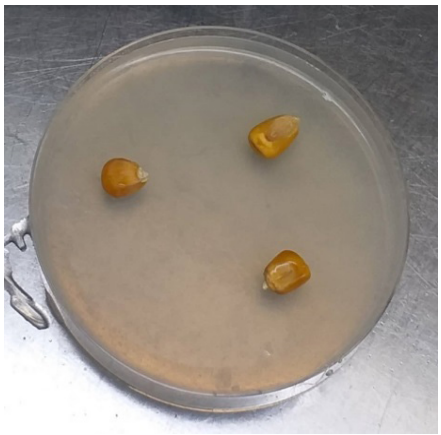


Figure 1: Seeding corn kernels on PDA.

Molds Identification

The isolated strains are first identified according to the cultural characters described by Djossou [11] and Chakranarayan et al. [12]. These cultural characters relate to the diameter, color and underside of the colony. In addition, cultural characters, culture fractions are examined by optical microscopy (Carl Zeiss, Oberkochen, Germany) on an ordinary slide: observation relates to the presence or absence of metulae, the shape of the vesicle, the appearance and color of the conidiophore and conidia [12-14].

To verify the aflatoxinogenic nature of the isolated Aspergillus strains, the latter are inoculated onto the selective culture medium AFPA (Aspergillus Flavus Parasiticus Agar).

The orange-yellow coloring of the reverse side of the colonies on the AFPA medium confirms the aflatoxinogenic character of the strains.

Statistical Analyzes

Statistical analyzes are performed with R software version 3.5.3. Standards for the distribution of aflatoxin concentrations in maize were calculated and tested by Kruskal-Wallis. P values less than 0.05 are considered significant.

Results

Aflatoxin Levels of Imported Maize

The total aflatoxin contents of imported maize according to the country of origin are presented in Table 1.

Table 1: Aflatoxin Levels by Country of Origin.

Sample (S)	Aflatoxin content (F1+F2+G1+G2) (ppb)		
	France	Argentina	USA
S1	2.3	2	2.2
S2	2.3	6.5	5.7
S3	6	2	2.3
S4	1.9	2.5	2.4
S5	2.7	2.8	3.1
S6	2.6	1.8	4.3
S7	4.05	4.3	2.2
S8	2.2	2.3	3
S9	3.4	1.9	4.1
S10	3.2	5.1	2.2
Average content (± standard deviation)	**3.07 ± 1.22**	**3.12 ± 1.62**	**3.15 ± 1.21**

Maize analysis results showed that all samples are contaminated with aflatoxins. The aflatoxin contents of the samples vary from 1.8 ppb to 6.5 ppb. Specifically, they vary from 1.9 to 4.05 ppb for France, from 1.8 to 6.5 ppb for Argentina and finally from 2.2 to 5.7 ppb for the United States. Aflatoxin content in all samples meets the US standard of 20 ppb. On the other hand, only 23 samples show aflatoxin levels below the maximum value of 4 ppb set by the European Union. 7 samples have grades above this value. France presents 1 sample that exceeds 4 ppb. As for Argentina and the USA, they have 3 samples each exceeding this value. Statistical analyzes show that there is no significant difference in average aflatoxin levels between countries (P = 0.07).

Mycotoxinogenic Molds

The main genera of potentially mycotoxigenic molds found on imported corn are Aspergillus and Penicillium.

Morphological Characteristics of Aspergillus

Among the strains of Aspergillus that have been isolated, two have been identified as potentially aflatoxinogenic: *Aspergillus flavus* and *Aspergillus parasiticus.*

For *A. flavus*, the colonies obtained have a diameter of 9 cm after 7 days of incubation. They are olive green and yellowish on the reverse side with a few white sclerotia on the surface and a radiant velvety texture (Figure 2a). Observation under an optical microscope of the isolated strain shows globose conidia and a non-chambered, hyaline and colorless conidiophore. The conidial head is radiating, uniseriate with a globose to sub-globose vesicle (Figure 2b).

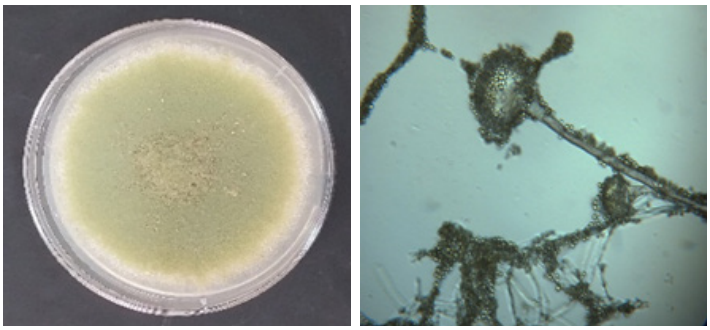


Figure 2a: Macroscopic appearance; **Figure 2b:** Microscopic appearance.

Concerning *A. parasiticus*, the colonies obtained have a diameter of 9 cm after 7 days of incubation. They are dark olive green in color, without sclerotia, and orange-yellow on the reverse side with a radiant velvety texture (Figure 3a). Observation under an optical microscope of the isolated strain shows globose conidia and a non-chambered, hyaline and colorless conidiophore. The conidial head is radiating, uniseriate with a globose to sub-globose vesicle (Figure 3b).

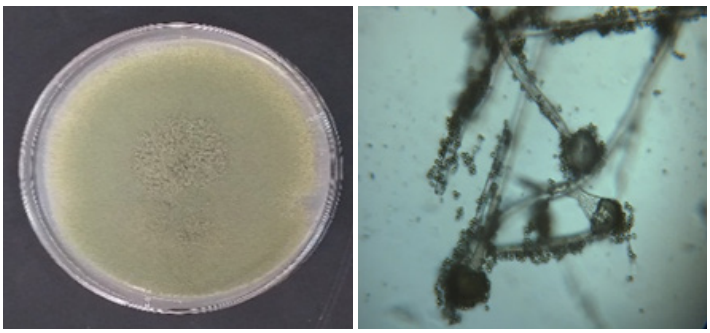
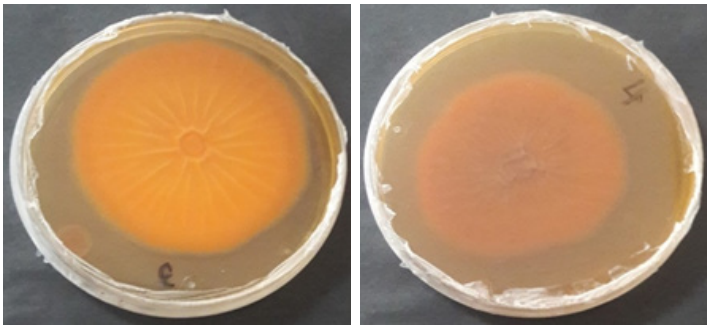


Figure 3a: Macroscopic appearance; **Figure 3b:** Microscopic appearance.

The orange-yellow staining of the reverse side of the colony (Figure 4) of these two Aspergillus on the AFPA medium confirms that the isolated strains of *A. flavus* and *A. parasicitus* are indeed aflatoxinogenic.



Reverse side *A. flavus* colony **Reverse side *A. parasiticus* colony**

Figure 4: aflatoxinogenic test of strains of *a. Flavus* and *a. Parasitucus* isolated on afpa culture medium.

Morphological characteristics of Penicillium

Isolated Penicillium strains have a powdery blue to green thallus surrounded by a slightly fluffy white part (Figure 5a). The asexual reproductive structures of Penicillium are in the form of a brush, sometimes bi-verticillated (Figure 5b).

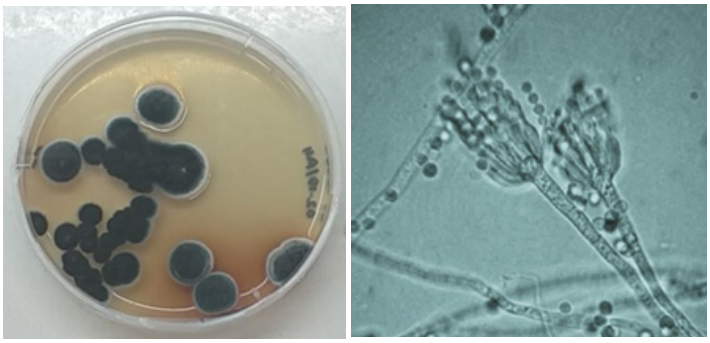


Figure 5a: Macroscopic appearance; **Figure 5b:** Microscopic appearance.

Discussion

The results show that 35% of the samples of maize imported into Senegal from France, Argentina and the United States have total aflatoxin levels that exceed the standards set by the European regulations in force. The average aflatoxin content of maize from these three countries is 3.1 ppb. Regular consumption of this corn can cause long-term chronic exposure. Indeed, according to Coppock [15], regular consumption of foods containing 2.5 to 3.5 ppb of aflatoxins can lead to chronic exposure. The latter promotes several diseases such as liver cirrhosis, cancer and hepatitis B [16-18]. In Senegal, this risk is greater in rural areas where maize consumption is three times higher than in urban areas [6].

The aflatoxin levels of imported maize are much lower than those of local maize. The latter can contain grades ranging from 8.0-1081ppb [19]. However, imported maize is often not immediately consumed. It is stored for days at the food industry level or for a longer period at the population level.

Given the inadequate storage conditions in the country, the aflatoxin content of imported maize is likely to increase with storage time. Duris [20] reports that poorly controlled storage

conditions and storage duration pose a major risk for the formation of mycotoxins. Humidity and temperature are the main physical factors having a considerable influence on the growth and production of mycotoxins [21].

The aflatoxin levels of maize sampled at industrial level are higher than those found in their country of origin. For example, in France the maximum content of aflatoxins in maize is 0.80 ppb [22] while we found an average content of 3.01 ppb. This difference would come from the storage conditions of the corn grains in the containers during its transport by boat and to a lesser extent by a storage of a few days at the industrial level. This shows the ability of aflatoxinogenic Aspergillus to rapidly secrete large amounts of aflatoxin when storage conditions are favorable.

The results of mycological analyzes of maize grains revealed contamination by potentially mycotoxinogenic molds: Aspergillus and Penicillium. These two kinds of molds that we have been able to identify are contaminants of mistreated but above all badly preserved foodstuffs, they are considered as storage contaminants of cereals and their derivatives [23]. They are able to alter the quality of grains by possibly producing mycotoxins such as aflatoxins, ochratoxins and patulin. Their development on cereals during storage is directly linked to hydrothermal conditions [24]. However, the presence of mycotoxinogenic molds does not necessarily mean the production of mycotoxins [25]. The conditions allowing mycotoxinogenesis are narrower than those allowing fungal growth.

The potentially aflatoxinogenic Aspergillus strains identified in our maize samples are *A. flavus* and *A. parasiticus*. The dominance of the genus Aspergillus in the contaminating flora of cereals has been reported in several studies [26,27]. Their negative impact lies in the alteration of substrates (organoleptic quality: taste, color, flavor, texture) and in the secretion of aflatoxins, including aflatoxin B1, which is the most formidable due to its carcinogenic, immunosuppressive and teratogenic effects [1]. The presence of aflatoxinogenic Aspergillus with that of the genus Penicillium heavily depreciates the quality of imported maize grains. Penicillium are potential producers of mycotoxins such as ochratoxins and patulin which have effects similar to those of aflatoxins [28-30].

Conclusion

This study showed that the total aflatoxin content of maize imported into Senegal varies from 1.8 ppb to 6.5 ppb. In 35% of the samples, the levels of aflatoxin contamination exceed the standards set by the European regulations in force. Mycological analyzes showed the presence of potentially mycotoxinogenic molds in this maize. These are two aflatoxin-producing Aspergillus (*A. flavus* and *A. parasiticus*) and *Penicillium sp*. These molds are capable of rapidly increasing mycotoxin levels in maize when conditions are favorable, particularly in storage areas. Thus, there is an urgent need to develop practical and economical methods of controlling mycotoxinogenic molds to prevent the secretion of mycotoxins particularly during the transport and storage of foodstuffs.

Acknowledgements

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