THE ISOLATION OF *ASPERGILLUS* SPP FROM HARVESTED MAIZE IN THREE PORTUGUESE REGIONS

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Introduction

In Portugal, maize (Zea mays L.) is the cereal that involves more agriculture explorations being a source of food, forage and processed products for industry. Fungal infection can result in mycotoxin contamination during growing, harvesting, storage, transport and processing (Chulze, 2010). Poor storage conditions of this commodity can expose it to factors such as high temperatures, condensation, insects and animals, leading to the reduction of its nutritional value, the development of toxigenic fungi and mycotoxin accumulation (Richard et al., 2009). To prevent fungal development it's important to study the production chain. *Aspergillus* spp. are usually associated with this cereal during drying and storage (Samapundo *et al*. 2007), making it susceptible to mycotoxins such as aflatoxins, ochratoxins, cyclopiazonic acid and fumonisins.

The aim of this study was to evaluate the mycotoxigenic potential of isolated *Aspergillus* strains from maize samples and correlate it with the sampling place and weather conditions.

Materials and Methods

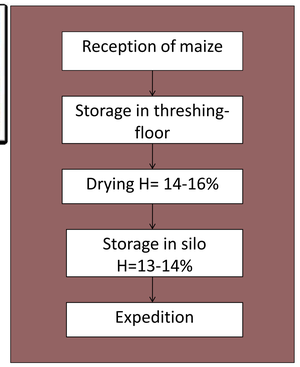
•The survey was carried out in three Portuguese regions between November 2008 and April 2009.

• The samples were collected from producers belonging to the National Producers Association of Maize and Sorghum (ANPROMIS – Associação Nacional de Produtores de Milho e de Sorgo):Cooperativa Agrícola de Coimbra (Coimbra); AGROMAIS (Riachos); CERSUL (Santa Eulália/Elvas).

•The sampling occurred in three distinct places of the storage chain (figure 1): reception; drying and expedition.

Sampling at reception

Maize dryer



Silos for maize storage

Figure 1. Fluxogram of the storage chain.

-Maize samples were plated in MEA10 (malt extract medium agar with 10% of NaCl) and the resulting fungi were isolated to MEA;

- Presence of mycotoxins was tested by high profile liquid chromatography (HPLC), by growing the *Aspergillus spp.* isolates in YES (for aflatoxins) and CYA (for OTA; CPA and Fumonisins)

Results and Discussion

○ It was possible to obtain, from a total of 132 samples, 1075 isolates from the gender *Aspergillus* and 732 of this isolates were tested for mycotoxins;

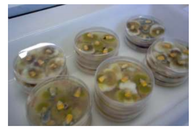
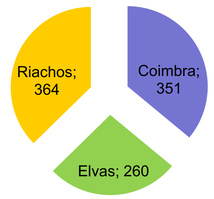


Figure 2- Plates of MEA10 with 5 grains of maize. Fungal growth after 10 days at 25^{0}C

○ Results show that there are differences between the incidence of the three groups of *Aspergillus* in the regions. Whereas in Elvas (Portalegre) and Riachos (Santarém) there is a high incidence of *Aspergillus* section *Nigri*, the same doesn't happens in Coimbra. This may be explained by the fact that black *aspergilli* are more resistant to the higher solar exposure and higher temperatures, typical of these regions (figure 2);

Riachos has a micro-climate, typically continental with Mediterranean influences due to the proximity of Air Mountains, inducing a very dry and hot climate during summer time.



Coimbra climate is dominated by Atlantic influences with high precipitation values and moderate temperatures.

Elvas climate is dominated by Mediterranean influences, being very hot and dry during summer time.

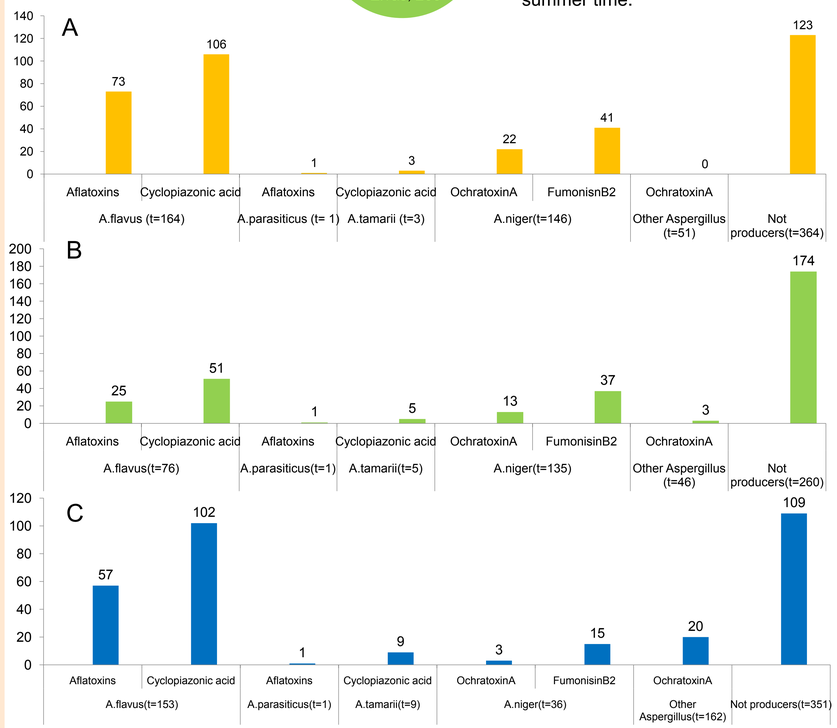


Figure 3. Number of *Aspergillus spp.* isolates obtained in the studied regions with the respective climate associated. A, B, C: number of isolates divided in distinct groups and their toxigenic potential, per region (t = total of isolates).

Results and Discussion

○ A very small percentage of *Aspergillus* section *Nigri* are OTA producers even though they are abundant in the samples of two regions. However, there is the occurence of fumonisins B2 production by some strains;

○ *Aspergillus* section *Flavi,* are more common in the samples after leaving the dryers. They have been devided in three groups of species: *A. flavus*, *A,parasiticus* and *A.tamarii*. Most of *A. flavus* are producers of CPA as well as all *A. tamarii.* The only *A. parasiticus* isolate is producer of aflatoxins as expected for this species and less than 50% of *A. flavus* are producers of aflatoxins.

Conclusion

It is possible to correlate the climate with the kind of isolates obtained, being *Aspergillus* section *Nigri* associated with the regions of hotter and dryer climates. *Aspergiilus* section *Flavi* are very common in all regions even though they are more common after drying and storage. The great majority of fungi are not producers, but there is an alarming quantity of producers of aflatoxins and CPA.

The fact that this strains can produce mycotoxins in ideal conditions doesn't mean necessarily that they can also produce in the original matrix (maize). Nevertheless, the mycotoxigenic potential of these fungi shouldn't be ignore because their occurrence means not only an health risk but also a big economical loss.

References

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