STUDY REGARDING THE IDENTIFICATION OF MYCOTOXIGENOUS FUNGI FROM THE SEED MASS OF MAIZE

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Abstract. *In the maize crops are present three fungi species from the genus Fusarium that produces micromicotoxins. First is Fusarium verticillioides sin. Fusarium moniliforme. The sexuate form of this fungus is Gibberella moniliformis known also as Gibberella fujikuroi. It is the fungus that produces the micromicotoxin "fumonisine". Fumonisine is often produced in field, but also it can be formed in the stored maize. The second fungus is Fusarium proliferatum implied also in the production of "fumonisine" and the third fungus is Fusarium graminearum that synthesises the micromicotoxins "trichotecen" and "zearalenon". The perfect form is Gibberella zeae. With the Fusarium species, in the maize crops can appear the fungus Aspergillus flavus too, major producer of mycotoxins names mycotoxins. Thus, if in the past was known that the fungi from Aspergillus genus attacks the maize during storage, nowadays is known that the infection appears in the maize crops in field. In the last years this fungus was present in the maize crops from Romania. The appearance of the fungus in the corn fields leads to the formation of the aflatoxins before harvesting. The implication of the mycotoxins in the quality and security of the plant products is recognized there being necessary their monitoring. The mentioned fungi cause problems in the dry years because it develops very well when the weather is hot during the night and dry during the day. Hail, drought, early frozen and the injuries caused by insects favour the infection. In the year 2015 there were isolated from maize seeds species of mycotoxigenous fungi together with other species pathogeniconly. During this research the isolation of fungi was done from the maize seeds sampled from a grain store from Semlac (Arad).The isolation was realised from the maize seeds' surface. On the seeds' surface from the wet chambers were developed fungi from the genera: Alternaria, Penicillium, Aspergillus, Fusarium, Helminthosporium. Predominant was growing the mycelium of Fusarium graminearum species. The obtained results show that the seeds fungal contamination rate (SCI) with micotoxigenous fungi was 44.33% for Fusarium graminearum and 4.33% for Aspergillus flavus.*

Key words: *Aspergillus flavus, Fusarium graminearum, contamination, maize, mycotoxins, seeds.*

INTRODUCTION

Maize is attacked by a great number of pathogens both in field and in store. Many fungi species are responsible by the maize grains damage during the storage. In stores are predominant the following fungi species *Fusarium sp.*, *Aspergillus sp.* and *Penicillium sp.* A research developed in Ethiopia shows that *Aspergillus sp.* is predominant in the maize grain mass being followed by *Fusarium sp.* (TSEDALEY B., ADUGNA G., 2016).

At worldwide level maize is on the third place as food source and as cultivated surface. On the first positions is wheat and rice (KYENPIA E. O. *ET AL.*, 2009). There is estimated that until on 2050 the surfaces cultivated with maize will increase because the demand for consumption is greater in the developing countries (ROSEGRANT M. W. *ET AL.*, 2008).

The most recent researches show that the most frequent isolated storage pathogens are *Aspergillus* and *Fusarium* followed by the *Penicillium* species (TSEDALEY B., ADUGNA G., 2016). From the *Fusarium* species the most frequent in maize fields and stores are determinate mainly three species.

First is *Fusarium verticillioides* sin. *Fusarium moniliforme*. The sexuate form of this fungus is *Gibberella moniliformis*, known also as *Gibberella fujikuroi.* This species produces the mycotoxin type named „fumonisine”. Fumonisine is most often produced in field, but also is developing well in the stored maize grains. The second *Fusarium* species is *Fusarium proliferatum* that is producing „fumonisine” too, and the third is *Fusarium graminearum* that is synthesizing the mycotoxind *"trichotecene*" and „*zearalenone*”. The perfect form of this fungus is *Gibberella zeae.* In the same time with *Fusarium*, in the maize field crops can appear *Aspergillus flavus*, also a great major producer of mycotoxins named aflatoxins. If in the past was well known that the fungi from *Aspergillus* genus attack maize during storage period, nowadays is known that the infection with this pathogen appears in the maize field crops too. The above mentioned fungi species are developing in the field crops mainly in the dry years, when the temperatures are during the night and dry during the day. The monitoring of these species is necessary due to the implication of the mycotoxins that are an important issue in the agri-food security and quality. The mycotoxins found in cereals and than in the food products affect the human and animal health (ABATE D., ABEGAZ B. G., 1985; BERCA M., 2003**;** GEYED A., MARU A., 1987).

The purpose of this research is to identify the myco-toxicogenous fungi from the maize grain mass collected from a cereal store from Semlac (Arad County). The stored maize was originating from field where the fungi *Fusarium sp*. and *Aspergillus flavus* where determined. The isolation was realised from the maize grain surface.

MATERIAL AND METHODS

The biological material used in this research was consisted in three maize seed samples collected from a cereal store from Semlac (Arad County).

The isolation of the fungi was done from the maize seed surface. There were extracted 100 maize grains from every sample. In a first phase the seeds were washed with tap water. After washing the seeds were disinfected by submersion in ethylic alcohol 96% for 1 minute. The next phase was consisting in successive washing of the disinfected seeds in two baths with sterile water. The drying of the washed and disinfected seeds was done on sterile filter paper. The dry seeds were placed with a sterile pincers (treated with flame) in Petri plates on the surface of the filter paper previously wetted (humid chamber).

The humid chamber method is simple and consists in the covering of the Petri plates with filter paper discs proper with the plate dimension. On the surface prepared in this way were placed the disinfected maize grains. Every sample had 100 grains and 3 replicates (Petri plates). This was necessary for the assessment of the percentage of the developed fungi. The Petri plates prepared as was mentioned above were kept in incubatory at a temperature of 20 - 22 ºC for 7 days.

The grain contamination rate with fungi (SCI) was calculated using the following formula (after DOOLOTKELDIEVA *ET AL*., 2010):

REZULTS AND DISCUSSIONS

After 7 days of incubation the seed samples were analysed visually at the binocular magnifier. Later on there were realised microscopic slides for an accurate identification of the fungi that have grown on the surface of the seeds placed in Petri plates. At the surface of the grains from humid chambers were developed fungi from the following genera: *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria* and *Helminthosporium* (figures 1, 2, 3 and 4).

Figure 1. Mycelium of *Aspergillus flavus* *(Photo: Otilia Cotuna)*

Figure 2. Mycelium of *Fusarium graminearum (Photo:Otilia Cotuna)*

Figure 3. Mycelium of *Alternaria sp.* *(Photo: Otilia Cotuna)*

Figure 4. Mycelium of *Penicillium sp*. *(Photo: Otilia Cotuna)*

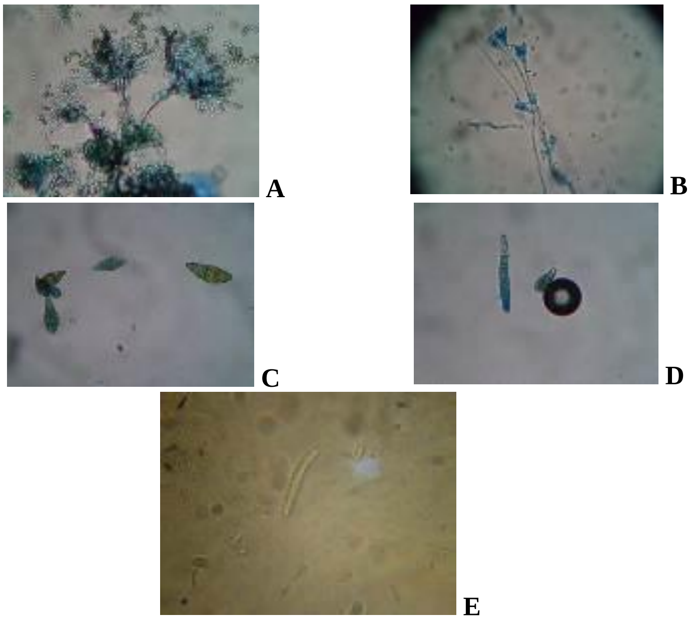

Figure 5. Conidiophores and conidia at microscope: A - *Aspergillus flavus*; B - *Penicillium sp.*; C - *Alternaria sp.*; D - *Helminthosporium sp.*; E - *Fusarium graminearum. (Photo: Otilia Cotuna, 2016)*

The examination at the microscope was compulsory for the accurate identification of the fungi developed on the maize grains from the humid chambers. The microscopic analysis has evidenced the presence of the conidia of the following fungi *Fusarium sp.*, *Alternaria sp.*, *Helminthosporium sp.*, *Aspergillus flavus* (fialids with conidia) and *Penicillium sp.* (figure 5).

Table 1

Results regarding the contamination of the maize grains from the humid chambers with the mycotoxicogenous fungi Aspergillus flavus and Fusarium graminearum

Fungus	No. of contaminated grains from 100			Total	Average	Contaminated/ non-contaminated rate
	R_1	R_2	R_3			
Aspergillus flavus	**5**	**6**	**2**	**13**	**4.33**	**4.33%**
Fusarium sp.	**35**	**56**	**42**	**133**	**44.33**	**44.33%**
Penicillium sp.	2	5	4	11	3.66	3.66%
Alternaria sp.	3	6	7	16	5.33	5.33%
Total	45	73	55	173	57.66	
Non-contaminated grains	55	27	45	127	42.33	42.33%

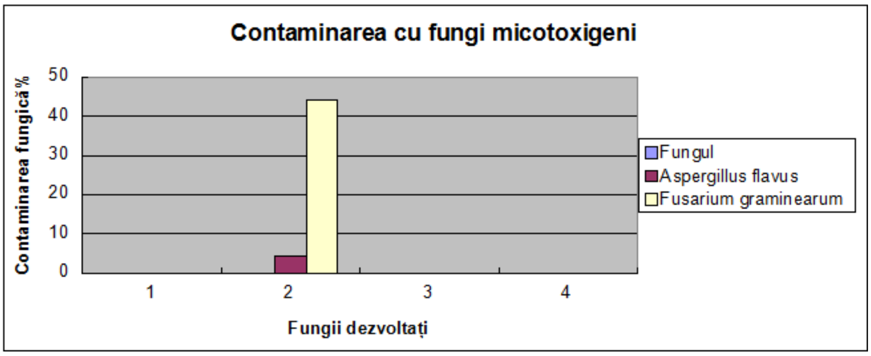

Figure 6. Contamination of the maize grains with mycotoxicogenous fungi

The results obtained in this research show that in the maize grain mass collected from the store from (harvest from the year 2015) are present species of mycotoxicogenous fungi. The greatest rate of fungal contamination was registered in the case of the pathogen *Fusarium*, respectively about 45%. Opposite is situated the infection with *Aspergillus flavus* with about 5% fungal contamination index. The first two pathogens are followed by *Penicillium sp*. with an incidence of about 4%. The presence of the fungi in the seed mass during the storage period can affect the quality and nutritive value due to the mycotoxins that are synthesized (RICHARD J. L., G. A. PAYNE, 2003). After quality there can be affected the germination rate of the seeds. As the fungal contamination is greater the germination rate of the seeds will be lower (QUEZADA *et al*., 2006). The economic loses that can appear due to the mycotoxigenous fungi can be great for the farmers, for animal breeders and for the processers (SHETTY P. H., R. V. BHAT, 1999).

The presence of the two mycotoxigenous fungi in the analysed grain mass is correlated with their presence in the maize fields, the climatic conditions of the year 2015 being very favourable to the attack. The monitoring of those pathogens extremely dangerous is necessary due to the implication of the mycotoxins in the security and quality of the vegetal products.

CONCLUSIONS

The results of this research confirm the presence in the maize grain mass of the mycotoxigenous fungi *Aspergillus flavus* and *Fusarium sp.* The greatest fungal contamination index was registered in the case of the fungus *Fusarium sp*., about 45%. The contamination of the maize grains with the aflatoxins producer fungus *Aspergillus flavus* was about 5%. The presence of the two mycotoxigenous fungi in the maize grain mass is in tide correlation with the presence of the fungi in the maize fields in the last years on the background of a dry climate favourable to the pathogeny.

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