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MYCOTIC AND MYCOTOXIC CHARGE EVALUATION OF WHEAT SEEDS FROM TRANSILVANIA AREA

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Abstract

This paper is willing to present preliminary results of the research (doctoral thesis) which has the main scope to evaluate the mycotic and mycotoxic charge of grains from Transylvania area. Current data are referring to the mycotoxic charge of wheat seeds harvested in 2005 from 9 districts from Transylvania area. As we know, mycotoxines are toxic metabolites of some fungus presents often at grains as warehouse characteristic micro flora.
Keywords: *wheat seeds, micro flora, mycotoxines, TLC, maximum levels.*

Introduction

Mycotoxines are toxic secondary metabolites represented by a large diversity of chemical substances, produced by some funguses during their development on crop plants, on seeds during storage, on feed or food products (Beattie, 2005). The funguses types that seem to produce constantly mycotoxines are *Aspergillus*, *Penicillium* and *Fusarium*.

Aflatoxines are fluvocumarinic substances produced by the funguses *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Penicillium puberulum, Rhizopus sp.* Not all the stalks of these species are toxic. Aflatoxines appear in nuts, oil seeds, cereals, legumes seeds, cocoa, spices, meat, milk and dairies (Laslo, 1995).

The optimum temperature of the aflatoxines elaboration is 20°C. 17 fractions are known: B_1, B_2, $B_{2\alpha}$, G_1, G_2, $G_{2\alpha}$, M_1, M_2, $M_{2\alpha}$, GM_1, GM_2, P_1, P_2, Q_1, Q_2, aflatoxicol, aspertoxin. The most important and toxic is

B_1. Its toxic effects expand upon a large variety of biological systems, plants, fish, batrachians, birds and animals.

Aflatoxines are dangerous because they resist to high temperature, usual disinfections, UV and X radiation treatments (http://193.132.193.215/eman2/fsheet2.asp).

Ochratoxins are products of the funguses *Aspergillus (A. ochraceus, A. candidus, A. fumigatus, A. sulfureus)* and *Penicillium (P. viridicatum)* and appear in corn, wheat, barley, oat, legumes seeds and green coffee beans.

They are nephrotoxic mycotoxines, involved in renal diseases in human and also in animals. There are three types: A, B and C. Ochratoxin A is the most representative quantitatively and 16 times more active then type B. It is a teratogenic and carcinogenic substance (http://193.132.193.215/eman2/fsheet3.asp).

In human beings it produces endemic nephritis, a chronic disease, frequent in rural areas and in women between 30 and 50 years old, presenting a high mortality.

Experimental

To analyze the mycotic charge of the wheat seeds we used the writing pad method (Raicu, 1978): each sample was analyzed in 3 variations, by laying 10 wheat seeds per variation, in Petri boxes on wet writing pad and by incubating them at 20ºC(± 2º) for 9-10 days. The analyses were made on each seed using the binocular and the optic microscope (Bobeş, 1983; Florian, 2001; Puia, 2003). The final results of each sample has been done by making the average of the 3 variations, and the mycotic charge of the seeds from one district by making the average of the samples (10-15 per district) from that district.

Taking in account these results and the cost of the analyses imposed by the isolation and identification of mycotoxines, we have chosen 20 samples, the most representatives: 2 samples from each district, those with the highest frequency in funguses producers of mycotoxines (*Penicillium, Aspergillus, Fusarium*) and 2 samples chosen randomly with the lowest mycotic charge (one from Covasna and one from Timis).

The first step in mycotoxines isolation and identification was to establish the most efficient work method. At its basis stood the protocol described by Braicu and her collaborators (2005), protocol that detailed a method using the chloroform extraction for the simultaneous detection of aflatoxines (B_1, B_2, G_1, G_2) and Ochratoxin A.

Once the protocol was established we were able to make the screening of mycotoxines from wheat samples. To do it, we prepared first the samples, taking 100 g wheat seeds (from each sample reestablished after the evaluation of mycotic charge) and milling them to powder. For the extraction we have used samples of 10 g.

Materials used:

- Solvents: chloroform, acetonitrile, acetone, toluene, ethyl acetate, methanol, petroleum ether, benzene
- Reactive: acetic acid, sulfuric acid, potassium chloride, anhydrous sodium sulfate, silica gel
- Filter paper: Whatman 4
- Equipments: sonication bath, UV lamp.

The extraction of mycotoxines has been done by putting 50 ml chloroform and 10 g powder wheat in a glass pot and their sonication for 10 minutes. Chloroformed extract was then washed with distillated water and then passed over the anhydrous sodium sulfate and filtered in vacuum using filter paper.

Results and Discussions

Micro flora appeared after the incubation has been composed by saprophyte funguses characteristic for grains, from which took part *Alternaria (A. alternata), Fusarium (F. roseum), Penicillium sp., Aspergillus (A. flavus, A. niger), Epicocum (E. purpurascens), Rhizopus sp.*

The fungus with the highest frequency in all districts was *Alternaria alternata* (reaching 66.67% in Maramures district). Even if *Rhizopus* and *Epicocum* have reached the frequencies of 30.75% and 22.27% in Timis district, in the other districts, the frequency was quite low. Between the fungus inculpated of producing dangerous mycotoxines, *Fusarium* had the higher frequency, reaching 11.21% in Sibiu district, followed by *Penicillium* with the highest frequency in

Cluj district (3.64%) and by *Aspergillus*, that reached 2% in Hunedoara district (figure 1).

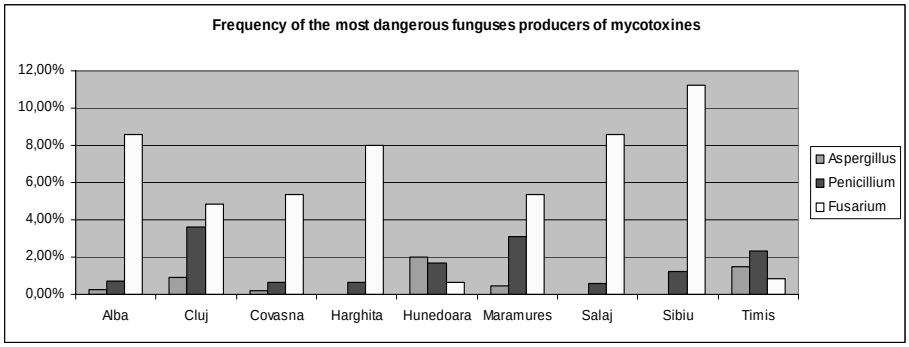

Figure 1. Frequency of the most dangerous funguses producers of mycotoxins in the wheat seeds from Transilvania area

The results of the mycotoxic charge evaluation of the wheat seeds are structured on each district and each mycotoxin isolated (aflatoxines B_1, B_2, G_1, G_2 and Ochratoxin A). The values of the mycotoxic contain are represented in table 1.

Table 1. Results of TLC analyses regarding the mycotoxic content (μg/kg) for each district from Transilvania area

District	Ochra-toxin A	Afla-toxin B_1	Afla-toxin B_2	Afla-toxin G_1	Afla-toxin G_2	Total Afla-toxines
Alba	4.5	3	1.5	0	0	4.5
Cluj	5.5	0	4	4.5	2	12.75
Covasna	1	1.5	2	1.16	1.16	5.83
Harghita	0	0	1.5	3.5	1.75	6.75
Hunedoara	2	0	0	4	5	9
Maramureş	4.5	1	1	1.75	1.75	5.5
Sălaj	0	0	0	0	0	0
Sibiu	1.25	0	0	1.5	1.5	3
Timiş	6.83	0	0	5.33	5.33	10.66

In conformation to the "Commission of Regulation" (EC), No 472/ 2002 from March 2002, amendment to the "Regulation" (EC) No

466/2001, the maximum level admitted for aflatoxine B_1 is 5 µg/kg, for total aflatoxines is 10 µg/kg, and for Ochratoxin A is 3 µg/kg.

From the total amount of the investigated samples, in 20% of the cases (belonging to Alba, Sălaj and Sibiu districts) it wasn't found any aflatoxine or Ochratoxin A, Sălaj district being the only one where it wasn't registered the presence of mycotoxines at all.

In 50% of the samples it was found Ochratoxin A, 40% from them (belonging to Alba, Cluj, Hunedoara, Maramureş and Timiş districts) having concentrations over the maximum level admitted. Taking in consideration districts averages, we can see that only 4 districts (Alba, Cluj, Maramureş and Timiş) are inculpated of exceeding the maximum level admitted by the law (figure no. 2).

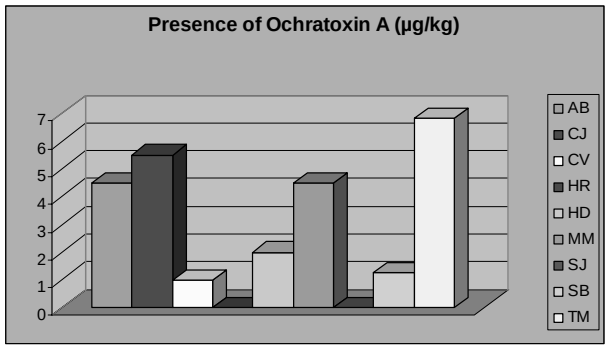

Figure 2. Presence of Ochratoxin A in the wheat seeds from Transilvania area

In 15% of the total samples it has been identified the presence of aflatoxine B_1, in 5% from them (belonging to Alba district) the concentration has exceeded the maximum level admitted. Taking in consideration the districts averages we can see that no district is incriminated of exceeding the maximum level admitted (figure 3).

In 35% of the total samples (belonging to Alba, Cluj, Covasna, Harghita and Maramureş districts) the aflatoxine B_2 is present, in 55% (belonging to Cluj, Covasna, Harghita, Hunedoara, Sibiu and Timiş districts) the aflatoxine G_1 is present and in 45% of the samples (belonging to Cluj, Covasna, Harghita, Hunedoara, Maramureş, Sibiu and Timiş district) the aflatoxine G_2 is present.

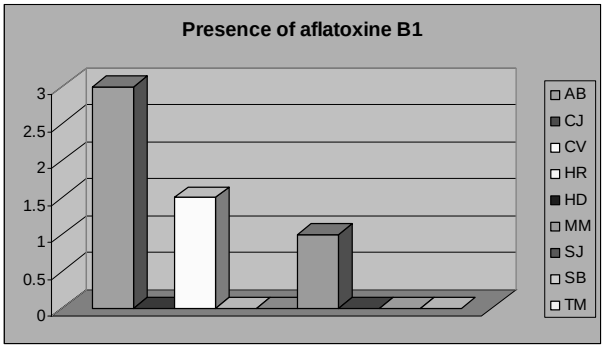

Figure 3. Presence of aflatoxine B1 in the wheat seeds from Transilvania area

From 70% cases in which total aflatoxines were found, only 15% of the samples (belonging to Cluj, Hunedoara and Timiş district) are exceeding the maximum level of 10 µg/kg admitted in grains.

Taking in consideration districts averages, we can see that only 2 districts (Cluj and Timiş) are incriminated of exceeding the maximum level admitted by the law (figure no. 4).

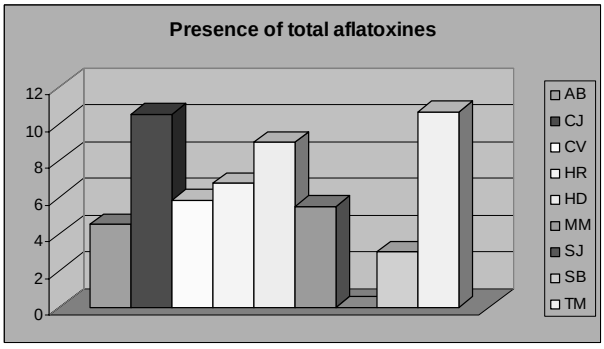

Figure 4. Presence of total aflatoxines in the wheat seeds from Transilvania area

If we are referring to those 2 samples free of funguses producers of aflatoxines and Ochratoxin A, we could reconfirm the idea of the remanence of the mycotoxines in the substrate after the fungus

disappearance (Măruțoiu, C., 2004), even in low quantities. If there was found aflatoxine B_2 in the sample from Covasna district, in a relatively low concentration, that doesn't impose risks (2 μg/kg), in the sample from Timiş district it was found the Ochratoxin A in a concentration that exceed much more the maximum level admitted by the law (8 μg/kg instead of 3 μg/kg), and the total aflatoxines concentration was quite near the maximum level admitted (8 μg/kg instead of 10 μg/kg).

Conclusions

Accordingly to the results of the analyses made on wheat seeds mycotoxic charge from Transylvania area on 2005, we have arrived to the following conclusions:

- The districts with the lowest risk are Sălaj and Sibiu, and those with the higher risk are Alba, Cluj, Maramureş and Timiş;
- Mycotoxines are products of funguses, substances that remain in the substrate even after their disappearance;
- It is necessary to continue the researches regarding the presence of mycotoxines produced by the other funguses encountered on the wheat seeds samples;
- It is necessary to continue the researches by applying a statistical method to correlate the mycotic charge of the wheat seeds with the appearance of the mycotoxines in that substrate and their concentrations.

References

Beattie, S. (2005). *The Impact of Fungi on Food Safety and Quality: Selected Cases*. "Food Safety in Agrofood Chain" Intenssive Programme, Gent

Bobeş, I. (1983). *Atlas de fitopatologie şi protecţia agrosistemelor*. Editura Ceres, Bucureşti

Braicu, C., Puia, C., Bele, C., Bodoki, E., Carmen S., (2005). Optimization of Screening Systems to Evaluate Relevant Mycotoxins From Cereals and Bread, *Buletinul USAMV Cluj-Napoca*, 61, 144-149

Florian, V. (2001). *Fitopatologie generală*. Editura Poliam, Cluj-Napoca

Laslo, C. (1995). *Elemente de ecotoxicologie*. Academic Press, Cluj-Napoca, p.32-40, 132

Măruțoiu, C., Tofană, M. (2004). *Analiza micotoxinelor*. Napoca Star, Cluj-Napoca
Puia, C. (2003). *Patologie vegetală*. Editura Digital Data, Cluj-Napoca
Raicu, C., Baciu, D. (1978). *Patologia seminței*. Editura Ceres, Bucureşti
http://193.132.193.215/eman2/fsheet2.asp
http://193.132.193.215/eman2/fsheet3.asp