INCIDENCE OF *FUSARIUM* SPECIES AND ITS MYCOTOXINS IN CEREALS FROM WESTERN ROMANIA

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Abstract: *Cereals are substrates favourable for the fungi development. The micromycetes proliferation can have undesired consequences: from the alteration of the aspect and of the nutritive features of the raw materials, incidence of mycoses or allergies till to the production and accumulation of mycotoxins. The goal of this study is to evaluate the presence of the Fusarium species in cereals from different areas of Banat region and to determinate the concentrations of deoxynivalenol and zearalenone, mycotoxins with a high frequency in the cereals from other regions of the country (south-east). Taking in account the Romanina climate, the fungal contamination is different in comparison with the one determined in other European countries. There were investigated mycologic and mycotoxicologic 56 samples of cereals (maize, wheat, barley and oat) collected from different areas of Banat. Fungal contamination degree was realised using the direct determination method for the total number of fungi colonies, and the mycotoxins concentration was determined with the immune-enzymatic method ELISA. The obtained results are similar with the ones obtained in investigations done in the south-east of Romania: the Fusarium species were present in all samples; the most frequent species were Fusarium graminearum and F. culmorum. Other Fusarium species identified there were: F. poae, F. oxysporum and F. vertiliciloides (F. moniliforme). From mycotoxicologic point of view the two analysed mycotoxins, deoxynivalenol and zearalenone, were identified in more than 90% of the samples and 70% of those were containing both mycotoxins. 25% from the analysed samples were containing deoxynivalenol in concentrations that were overpassing the levels allowed by EU (1750 µg/kg), and 40% from samples were containing zearalenone concentrations greater then 100 µg/kg, the maxim level allowed by EU.*

Key words: *Fusarium, cereals, deoxynivalenol, zearalenone, fumonizine*

INTRODUCTION

Fusarium genus comprises endo-parasite fungi species that are developing on cereals, fruits and legumes producing white-cream coloured, yellow, pink, and red until to violet moulds, present mainly in the soils from temperate regions.

Most of the *Fusarium* species are components of the soil micro-flora and they have a great geographical spread. *Fusarium* species are plant pathogens determining the rot of roots, stems and fruits, as well as the degradation of the vascular system. Many species are pathogen for humans and animals. Thus, many species are known as producers of mycotoxins from the trichotecenes group, zearalenones and fumonizines families, all of them with great implications in intoxication cases of humans and animals.

Some researches developed at INCDBNA Baloteşti have shown that in the maize designated for the combined forages processing and in the combined fodders for poultry, the *Fusarium* species were present in 80% and respectively 53% from the analysed samples, and the concentration of mycotoxins (deoxynivalenol and zearalenone) were presenting concentrations of 0.44-109.7 µg/g respectively 56.3-246.2 µg/g (TABUC *et al.*, 2004, TABUC, 2007).

Deoxynivalenol (DON or vomitoxine) is produced mainly by *Fusarium graminearum*

and *F. culmorum* that contaminate preferentially the cereals producing this mycotoxine. DON can be produced by other *Fusarium* species, too, as are: *F. crookwellense, F. sporotrichoides, F. poae, F. trichinctum* and *F. acuminatum.* DON presence in cereals determinates intoxications characterised by food refuse, nausea and vomiting, diarrhoea leading to drastic loses of weight till to the death of the animal by dehydration. These symptoms were noticed on swine (POLLMAN *et al.*, 1985; TRENHOLM *et al.*, 1984; HARVEY *et al.*, 1989), lambs (HARVEY *et al.*, 1986), meat poultry (HAMILTON *et al.*, 1985; HUFF *et al.*, 1981; HUFF *et al.*, 1986; KUBENA *et al.*, 1985; KUBENA *et al.*, 1989; SWAMY *et al.*, 2002a), ducks (BOSTON *et al.*, 1996) and turkeys (HAMILTON *et al.*, 1985; MORRIS *et al.*, 1999). Practically all animal species are sensitive to the action of this toxin, but toxicity differs from a species to another.

Zearalenone (toxin F2 or ZEN) is produced by de *Fusarium graminearum, F. semitectum, F. equiseti, F. crookwellense, F. culmorum, F. tricinctum, F. oxysporum, F. sporotrichioïdes* and *F. laterium.* Cereals contamination with zearalenone is a worldwide phenomenon because *Fusarium* species producing zearalenone can develop in all climatic types. ZEN determinates intoxications in many animal species as: swine (SWAMY *et al.*, 2002; YOUNG *et al.*, , 1990; GREEN *et al.*, 1990), bovines (DIEKMAN, GREEN, 1992), lambs (HUFSTEDLER *et al.*, 1996), meat poultry (SWAMY *et al.*, 2002b; CHI *et al.*, 1980), horses (MINERVINI *et al.*, 2006), lab rodents (YANG *et al.*, 2006; PEREZ-MARTINEZ *et al.*, 1997). The toxic effect of zearalenone is characterised through hyper-estrogenism and changes of the reproducing apparatus. Zearalenone is implicated in cases of hepato-toxicity, hemato-toxicity and immuno-toxicity (ABID ESSEFI *et al.*, 2004; CREPPY, 2002; MAAROUFI *et al.*, 1996; COULOMBE, 1993; COE *et al.*, 1992; HUSSEIN, BRASEL 2001, ZINEDINE *et al.*, 2007). Some toxic effects connected with oesophagitis and oesophageal cancer were reported in humans after the consumption of contaminated cereals in China and South Korea (LUO, 1990).

Fumonizines represent a group of mycotoxins characterised at the end of 80s, produced by *Fusarium verticillioides*, a mould species present worldwide and identified frequently in maize, sorghum and oat. These toxins can be synthesised by other species, too, as: *F. proliferatum* and *F. nygamai* sorghum and millet parasites, *F. oxysporum* and *F. polyphialidicum*, and by representatives of other genera as *Alternaria alternata sp. lycopersi*. Fumonizines toxicity is characterised by very different clinical signs, depending by species: in horses determinates equine leuko encephalomalacy (ELEM) characterised by the liquefying of the cerebral white substance, histo-pathologic abnormalities of the liver and kidneys (BAILLY *et al.*, 1996); the experimental administration of *F. verticilloides* in swine determinates lungs oedema (OSWEILER *et al.*, 1992; ROSS *et al.*, 1992); smaller doses of FB1 determinate acute liver toxicose (OSWEILER *et al.*, 1992 ; COLVIN *et al.*, 1993); also, FB1 is responsible by morpho-physiologic alterations of pancreas, heart, kidneys, oesophagus and alveolar endothelium. Fumonizines have immuno-toxic action characterised by the diminishing of the lymphocytes proliferation and the diminishing of the synthesis of some immunoglobulins (OSWEILER *et al.*, 1993, ROTTER *et al.*, 1996; MARTINOVA, MERRILL, 1995). In humans FB1 seems to be implied in oesophageal cancer observed in some countries from Africa and in China (YOSHIZAWA *et al.*, 1994).

This work is looking to evaluate the contamination degree of the cereals from the south-western Romania with *Fusarium* species and their toxins (deoxynivalenol, zearalenone and B1 fumonizine).

MATERIAL AND METHODS

The material used in this research is represented by 56 cereals samples (25 maize samples, 12 wheat samples, 10 barley samples and 9 oat samples) collected from different areas of Banat region. There were used Veratox kits for the determining of the mycotoxins

concentrations using the immuno-enzymatic method ELISA. The methods used are: isolation and identification of *Fusarium* species and determination of the mycotoxins concentration.

Isolation and identification of *Fusarium* species: *Fusarium* species are endophytes that contaminate the cereals in field during the ear formation. For the isolation of *Fusarium* species the cereal grains were washed with a solution of sodium hypochlorite and distilled water for the removal of the mould species that are developing on the grains' surface (*Aspergillus, Penicillium, Mucor, Rhizopus, Absidia, Cladosporium etc.*) ant that generally have a faster development invading the culture environment and inhibiting the development of *Fusarium* species. After washing and drying on filter paper the grains were sectioned with a sterile blade and deposited in Petri double disks ø 10 cm with Czapek environment. The disks were incubated for 5-7 days at 25°C. Through successive changes on PGA environment (potato-glucose-agar) there were obtained pure stands that were then used for the species identification. Identification was done through the study of the developed colonies on the base of their cultural, macroscopic and microscopic features described by NELSON *et al*. (1983) and BOTTON *et al*. (1990).

Determination of mycotoxins concentration: For the determination of the mycotoxins present in the cereal samples studied here were used Veratox Neogen kits for the mycotoxins dosing through immunoenzymatic method ELISA. At the determination of the mycotoxins concentration there were respected the stages described in the use instructions of every kit. The reading of the optical density was done during 10 minutes using a TEKAN microplates reader with the 650 nm wavelength and then was calculated the mycotoxins concentration.

RESULTS AND DISCUSSIONS

Isolation and identification of *Fusarium* species

The species of *Fusarium* genus were presented in all analysed samples; the most frequently identified species were *Fusarium graminearum, F. culmorum, F. poae, F. oxysporum* and *F. verticillioides* (figure 1). The results obtained are similar from the point of view of the microflora composition with the ones obtained in the studies done on the cereal samples coming from the south-east of Romania: *Fusarium graminearum* and *F. culmorum* are the most spread species, present in constant way in maize and in similar proportions (64.28% and respectively 62.50%); *Fusarium poae* and *F. oxysporum* present a greater frequency in south-west (32.14% and respectively 35.71%) in comparison with the south-east of the country where rarely is passing over 20%; regarding *F. verticillioides*, this species was identified in 37.5% from the studied samples, a great frequency in comparison with the cereals from the south-east where this species is rarely isolated, mostly on maize.

Determination of mycotoxins concentration

Maize: deoxynivalenol was determined in all 25 samples (100%). In 14 samples (56%) DON concentration overpasses the limit allowed by EU (1750 µg/kg). The greatest DON concentration was 2010.55 µg/kg. Zearalenone was also identified in all analysed maize samples, and 22 samples from the total 25 were containing ZEN concentrations greater then 100 µg/kg, the maximal allowed level. The greatest determined concentration was 212.7 µg/kg. Fumonizine was identified in 11 samples (44%) from the 25 analyse, but the determined concentrations were low, being comprised between 7.3 and 76.6 µg/kg. All studied samples were containing DON and ZEN, and 11 samples (44%) were containing all the 3 mycotoxin types produced by *Fusarium* genus. The concentrations of the *Fusarium* mycotoxins determined in maize are presented in figure 2.

Wheat: from those 12 wheat analysed samples 10 samples (83.33%) were containing DON in low concentrations (6.10 – 154.30 µg/kg), and 6 samples (50%) were containing

zearalenone in moderate concentrations comprised between 36.70 and 67.30 µg/kg. In none wheat sample were identified B1 fumonizine. 50% from the analyzed samples were containing both deoxynivalenol and zearalenone. The concentration of the *Fusarium* mycotoxins identified in wheat is presented in figure 3.

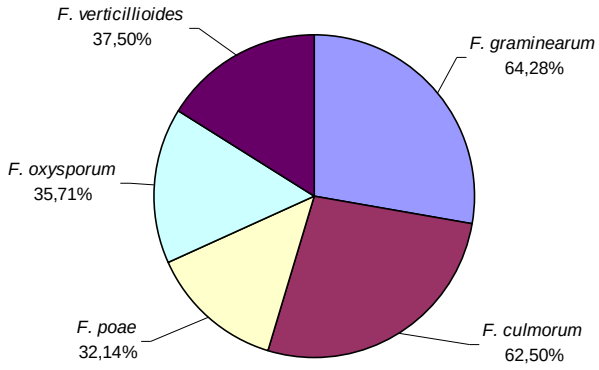

Figure 1: Isolated *Fusarium* species

Barley: from the analysis of the mycotoxins of barley samples there was noticed that all of them were containing DON, but the determined concentrations were between the limit levels allowed by EU legislation. Zearalenone was identified in 5 samples (50%) in low concentrations comprised between 26.25 and 546.50 µg/kg. In none from the 10 barley analysed samples was confirmed the presence of B1 fumonizine. 50% samples were containing both DON and ZEN. The concentration of the *Fusarium* mycotoxins identified in barley is presented in figure 4.

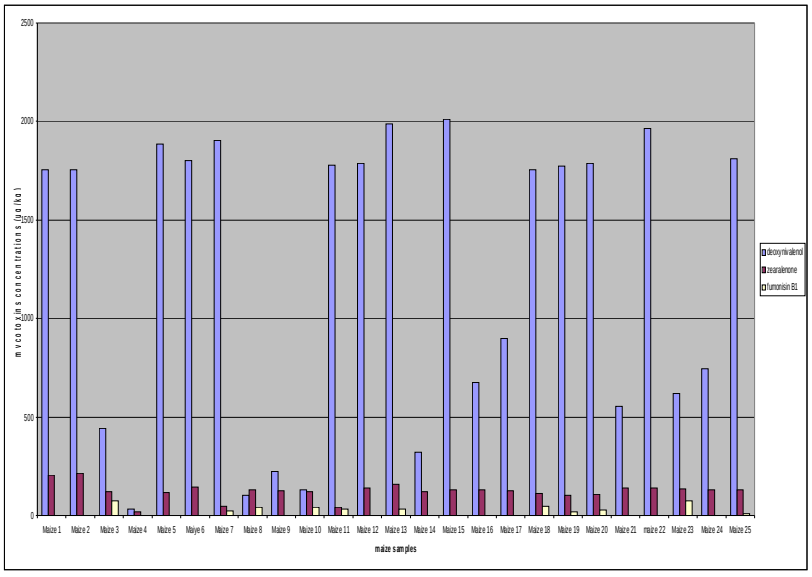

Figure 2: *Fusarium* mycotoxins concentration in maize samples

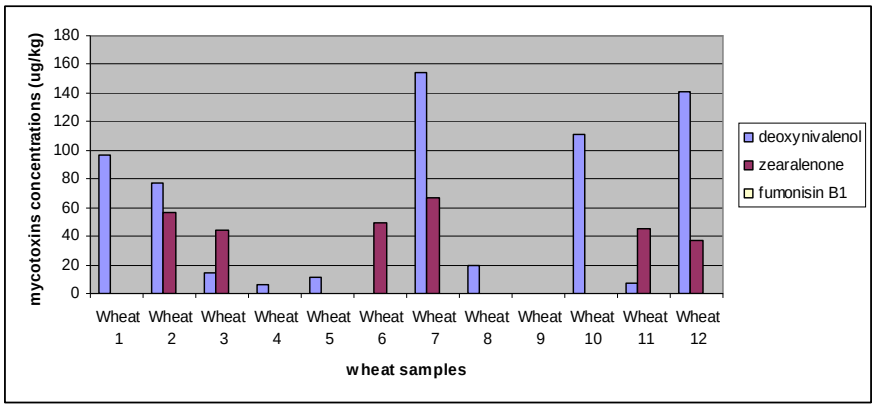

Figure 3: *Fusarium* mycotoxins concentration in wheat samples

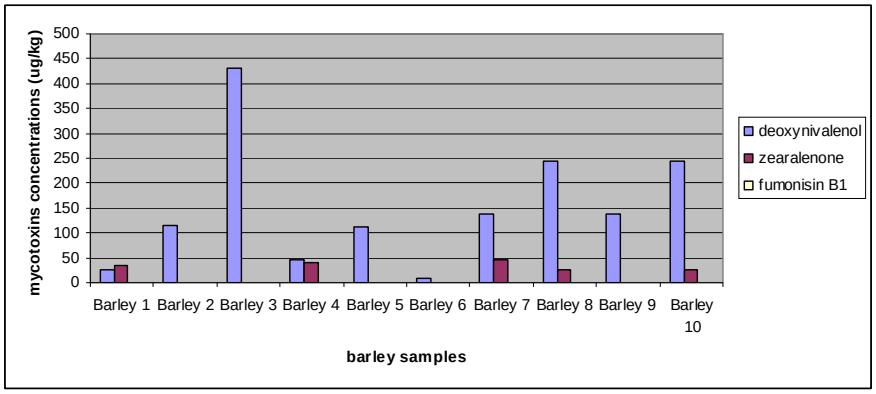

Figure 4: *Fusarium* mycotoxins concentration in barley samples

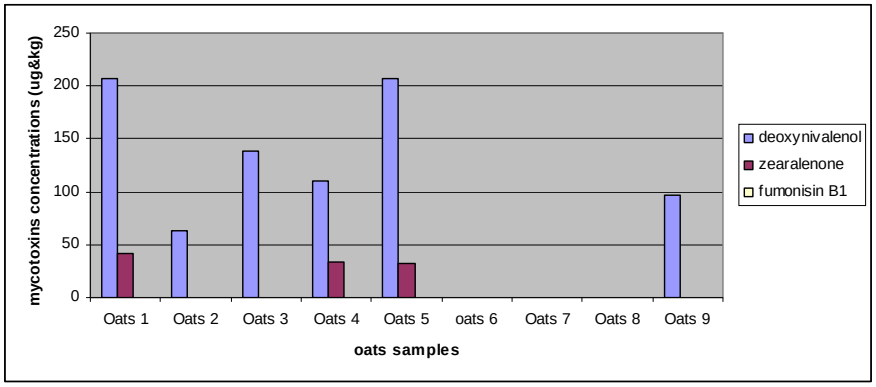

Figure 5: *Fusarium* mycotoxins concentration in oat samples

Oat: the analysis of the 9 oat samples has shown the presence of deoxynivalenol in 6 samples (66.66%) in low concentrations comprised between 62.70 and 207.3 µg/kg.

Zearalenone was identified in 3 samples (33.33%) in concentrations comprised between 31.6 and 41.6 μg/kg. The presence of B1 fumonizine hasn't been confirmed in any of the studied samples. From the total of the oat samples 3 were containing both deoxynivalenol and zearalenone. The concentration of the *Fusarium* mycotoxins identified in oat is presented in figure 5.

CONCLUSIONS

All the 56 cereal samples analysed were contaminated with *Fusarium* speciea; the most frequent isolated species were *F. graminearum* and *F. culmorum*; these results are in accordance with the ones obtained from similar studies done on the cereals cultivated in south-eastern Romania, were these two species are present constantly mainly in maize.

Maize is the most exposed cereal to the contamination with *Fusarium* species and the mycotoxins synthesised by them, this fact being confirmed by the results obtained in the south-east of the country, too; all the 25 maize analysed samples were contaminated with *Fusarium* and its mycotoxins. Deoxynivalenol and zearalenone were identified in all 25 maize samples, and 44% from these samples were containing all the three types of *Fusarium* mycotoxins. The 14 samples with DON concentrations higher then 17590 μg/kg and the 22 samples with ZEN concentrations greater then 100 μg/kg are belonging to maize samples.

From the 56 cereal samples analysed here, 51 samples (91.07%) were contaminated with DON, 39 samples (69.64%) with ZEN and 11 samples (19.64%) with B1 fumonizine. About 70% from the total of the analysed samples were containing two mycotoxins (deoxynivalenol and zearalenone) and about 20% were containing all the three *Fusarium* mycotoxins. Only in 4 samples from the 56 studied wasn't confirmed the presence of the analysed mycotoxins.

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