THE FUNGAL LOAD EVALUATION OF SOME WHEAT VARIETIES KERNELS IN STORAGE CONDITIONS

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Abstract

Mycotoxins are toxic secondary metabolites synthesized by fungal and developed in different substrates. Fungal contamination in cereals, their development and mycotoxin production can be produced in the field, during storage or in both periods. The main known mycotoxins that produce severe intoxications on humans and animals are synthesized mainly by 5 important genera of fungi such as: *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps purpurea*. The main objective of this research is to identify and describe micromycetes that develop on wheat kernels under storage conditions. Also, the frequency attack of Black Point disease was determined and the mycoflora involved on the analyzed varieties. The studied material is represented by the following wheat varieties: Izvor, Glosa, Antonius, Litera, Arieşan and Altigo. After the phytosanitary analysis the indentified genus of fungus were: *Penicillium, Fusarium, Aspergillus, Alternaria, Rhizopus* and *Trichothecium* and the frecquency of the mycromycetes differed from each studied variety.

Key words: micromycetes, wheat, black point

The importance of the wheat crop requires a special attention on the research regarding the health of plants and, especially, on mycoses that affect the crop. Thus the knowledge on the mycoflora of wheat varieties contributes to the number expanding of varieties that can be grown (Balau A.M. *et al*, 2014).

Also the study of black point disease on wheat helps the manufacturers to understand the attack mode and it's implications for achieving quality indicators of wheat seeds and the attack frequency in certain areas.

After harvest degradation of the agricultural products can be caused by many factors and at storage the production losses due to the activity of microorganisms can reach up to 20%.

The main fungal that develop on wheat kernels and produce mycotoxins are species belongign to the genus: *Aspergillus, Penicillim* and *Fusarium,* but alongside those there are genus like: *Rhizopus, Trichotecium, Alternaria, Trichoderma, Myrotecium, Stachybotys, Cephalosporium* and *Claviceps purpurea* (Miller J.D., Trenholm H.L., 1994).

Most of these micromycetes are saprophytic fungal and specific for storage conditions, except the *Fusarium* genus and *Claviceps purpurea* who are species that cause diseases in the field.

MATERIAL AND METHOD

The studied material consists of six winter wheat varieties that have been grown in different areas. The varieties Izvor, Glosa, Antonius, Litera Ariesan and Altigo constituted a total of 12 samples and were provided from different locations. To determine the fungal load of the wheat seed, the moist chamber method has been used (Mathur S.B, Kongsdal O., 2003). For each sample 400 of kernels were analyzed after six month of storage. The seeds were germinated in pleated filter paper at a distance of 1.5 cm, were then moistened with distilled water and kept in the moist chamber at 22℃ for 7 days (Hatman M. *et al*, 1986). The incubator was desinfected priorly and the spatula needle used for de prelevation of the mycelium developed on the wheat kernels was desinfected with alchool before each use.

Micromycetes were analyzed under a microscope for identification, and their determination the on the surface of the seeds was based on the characteristics listed in the speciality literature (Ellis M. B., Ellis P. J., 1985).

RESULTS AND DISCUSSIONS

Analysed results were structured for each sample separately and the identified micromycetes belong to the genus *Aspergillus, Penicillum, Fusarium*, *Alternaria, Trichotecium* and *Rhizopus*

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(*figure1*).

Aspergillus genus includes about 200 species of which *A. flavus, A. parasiticus, A. ochraceus, A. candidus, A. fumigatus, A. sulfureus, A. terreus, A. versicolor* produce mycotoxins (Summerbell R.C. *et al*. 1992).

Figure 1 **Fungus mycelium (*Fusarium* sp. *Alternaria* sp., *Aspergillus* sp., *Penicillum* sp.) developed on wheat kernels**

Regarding the analyzed samples the *Aspergillus* genus was identified with a frequency range between 0.5% on the Altigo variety (Pb12) and 4% on the Litera variety (Pb5).The fungi of the genus *Penicillium* are commonly found in grain storage and develop alfatoxins, ochratoxins and patuline. Except the Ariesan variety (Pb11), all kernels examined were identified with species from the *Penicillium* genus. This fungus formes co nidiophores from hyphae that branches digital forming a large verticiles resembling a skeleton hand. The highest value on the fungus frequency was recorded on the Glosa variety (Pb4), a value of 21.25% (*figure 2*).

Fusarium is one of the main macromycetes genera that secrete mycotoxins, the infections can start in the field and develop even in grain deposits. The mycelium of the genus *Fusarium* fungi has been identified in all samples. The highest value of the fungi frequency was recorded on Altigo variety and was by 77,5%.

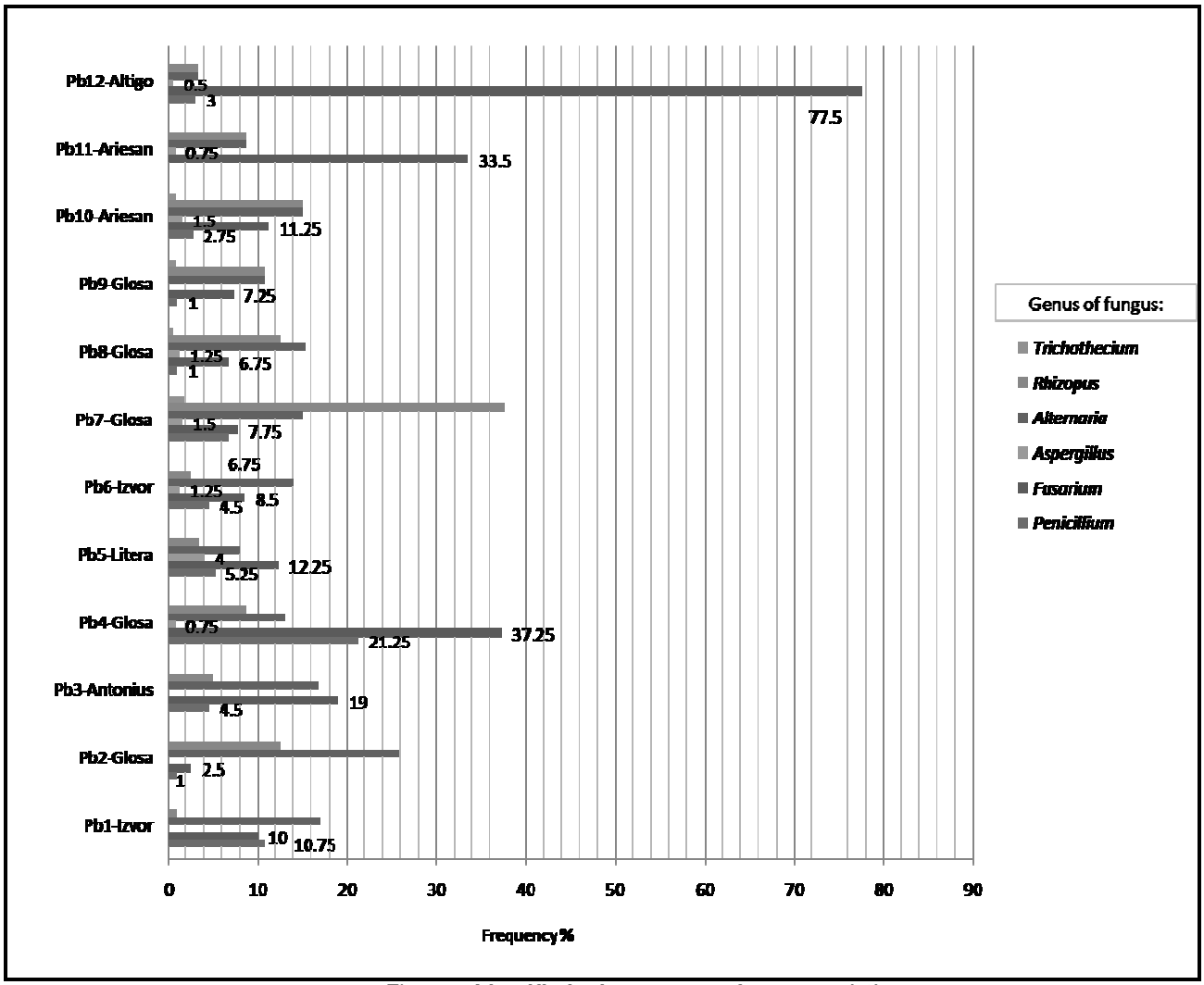

Figure 2 **Identified micromycetes frequency (%)**

Samples 11 and four represented by Ariesan and Glosa varieties also showed high values, over 30% of the micromycetes frequency from *Fusarium* genus. The lowest values were recorded on sample 4 by Glosa variety.

Fungus from the *Alternaria* genus contribute to black point appearance, but this disease is not harmful for wheat seed because it does not greatly harm the germination (Morar O.A., 2009). *Alternaria* sp. has been identified in all the studied wheat samples. The fungus forms a mycelium consisting of septate hyphae. Conidia are oblong, claviforme, caught in chains, light brown, longitudinal septated and cross through numerous septa. Although the highest percentage was recorded on Pb3-Antonius, Pb2-Glosa and Pb8 Glosa the blak point phenomenon was more common on kernels from Pb6-Izvor and Pb9-Glosa samples.

After a period of seven days incubation a higher value of germination was recorded on the free black point seed than on those affected (*table 1*).

Table 1

Characteristics of wheat seed germination affected by black point

Variety/ Sample	Black point attacked caryopses (%)	Germination (%) after 7 days
Pb1-Izvor	-	99
Pb2-Glosa	-	98.75
Pb3-Antonius	-	98.75
Pb4-Glosa	1.75	93
Pb5-Litera	9.25	98.25
Pb6-Izvor	29.5	98.75
Pb7- Glosa	5	98.25
Pb8-Glosa	3	98
Pb9-Glosa	27.5	99.75
Pb10-Ariesan	3.75	97.25
Pb11-Ariesan	0.75	98.75
Pb12-Altigo	2.25	99

The most common species of the genus *Rhizopus* in cereals contaminated by aflatoxin are *Rhyzopus nigricans* and *Rhyzopus stolonifer*. *Rhizopu*s sp. was easily spread and covered the whole seed and the paper fell seam from the incubation chamber. Thus, in all the analyzed samples this genus was identified and the highest percentage was recorded on Pb7- Glosa.

Along the fungi from the *Fusarium* genus, the *Trichothecium* genus are incrimitate to produce trichothecenes. *Trichothecium roseum* fungus is known as saprophytic on many substrates and occasional as weak parasite. On wheat kernels a pink mycelian felt appeared at first sight as the spore masses produced by *Fusarium* and *Gliocladium* was formed. But, after the microscopic analysis long conidiophores, thin, and hyaline septum of the *Trichothecium roseum* fungus were distinguished. Also conidia were formed in chains at the end of conidiophores, smooth, hyaline, ovoid, with a septum of 13-22x6,5-11µm.

CONCLUSIONS

The pathogens with the highest frequencies were from *Penicillium* and *Fusarium genus.*

The black-point attack did not reach high values although the *Alternaria* genus was identified in all the samples.

The presence of the fungus from *Aspergillus, Penicillium, Fusarium*, *Alternaria, Trichotecium and Rhizopus* genus represents a problem in terms of safety food because they produce mycotoxins. Therefore a permanent research on the fungal load of wheat kernels is required.

The development of the micromycetes that can produce mycotoxins is favored both by the climate conditions of a particular region and by the improper storage conditions.

ACKNOWLEGMENTS

This paper was published under the frame of Funding research projects contract – no. 5529/25.04.2013, USAMV "Ion Ionescu de la Brad" from Iasi.

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