MYCOLOGICAL INVESTIGATIONS REGARDING THE DOMINANT FLORA FROM THE VEGETAL SUBSTRATES HARVESTED FROM N-E ROMANIA

INVESTIGAȚII MICOLOGICE PRIVIND FLORA DOMINANTĂ DIN SUBSTRATURILE VEGETALE RECOLTATE DIN N-E ROMÂNIEI

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Abstract. *The research aimed to evaluate the fungal potential of plant substrates that frequently enter the food of animals and humans through a mycological screening of collected samples that characterize N-E Romania. Samples such as alfalfa semi-hay, corn silo, mixed feed ration, concentrates, corn grains were randomly collected from agricultural or zootechnical farms and a series of 30 determinations/sample were performed, in in order to establish the fungal load during the experimental period. The double agar serial dilution technique and bacterial inhibitors were used to establish the number of colony-forming units, with an emphasis on the isolation, identification and characterization of micromycete species, in view of their taxonomic classification. Following the study, it was shown that the dominant fungal flora that characterizes the plant substrates in N-E Romania belongs to the genera Penicillium (99.9%), Fusarium (89.6%) and Aspergillus (73%), the most contaminated samples being represented by the corn silo and the corn grains. The climatic conditions in this geographical area are favorable for these species of micromycetes, they develop a vigorous vegetative apparatus with a phenotypic expression difficult to confuse and easy to frame taxonomically.*
Key words: contamination, fungi, plant substrates, taxonomic classification.

Rezumat. *Cercetările au avut drept scop evaluarea potențialului fungic din substraturile vegetale care intră frecvent în hrana animalelor și a omului printr-un screening micologic din probe colectate ce caracterizează N-E României. S-au recoltat și analizat probe precum semifân de lucernă, siloz de porumb, rație mixtă furajeră, concentrate, boabe de porumb în mod randomizat din ferme cu profil agricol sau zootehnic și s-au efectuat o serie de 30 de determinări/probă, în vederea stabilirii încărcăturii micotice pe parcursul perioadei experimentale. S-a utilizat tehnica diluțiilor seriate în strat dublu de agar și inhibitori ai bacteriilor pentru stabilirea numărului de unități formatoare de colonii, accentul fiind pus și pe izolarea, identificarea și caracterizarea speciilor de micromicete, în vederea încadrării lor taxonomice. În urma studiului realizat, s-a demonstrat că flora micotică dominantă ce caracterizează substraturile vegetale din N-E României aparține în cea mai mare parte genurilor Penicillium (99,9%), Fusarium (89,6%) și Aspergillus*

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(73%), probele cele mai contaminate fiind reprezentate de silozul de porumb și boabele de porumb. Condițiile climatice din acest areal geografic sunt favorabile acestor specii de micromicete, ele dezvoltând un aparat vegetativ viguros cu o expresie fenotipică greu de confundat și ușor de încadrat taxonomic.

Cuvinte cheie: contaminare, fungi, substraturi vegetale, încadrare taxonomică.

INTRODUCTION

In agricultural or zootechnical units it is necessary to pay attention to plant substrates in terms of production, harvesting, conditioning and storage, in the sense that they must be of good quality when introduced as component parts in human and animal feed. Under certain climatic conditions, the vegetal substrates do not correspond entirely from a qualitative point of view, being attacked by different molds, even in the vegetative phase (Pfordt *et al*., 2020).

Micromycetes endowed with mycelial apparatus live in the soil or on plant products, thus, in their natural habitat, these micromycetes pour enormous amounts of spores, which are carried by the wind ensuring their permanent presence in the atmospheric air (Munkvold, 2017). For example, spores of the genus *Penicillium* are ranked third as the incidence among atmospheric spores. *Aspergillus* spores have been shown to rank fourth as aerogenic fungal flora after *Alternaria, Cladosporium* and *Penicillium* species (Mohale *et al*., 2013). Contamination of plant substrates with micromycetes is favored by the existence of optimal environmental conditions for their development, with negative consequences on agricultural production, animal husbandry and human, animal health (Oldenburg *et al*., 2017). Typically, mycotoxin-producing micromycetes that contaminate plant substrates are divided into two groups: "field" fungi that infect grains during field plant development (*Fusarium*) and "deposit" fungi that contaminate grains during storage (*Aspergillus, Penicillium*) (Khodaei *et al*., 2019). However, this classification is not strict because it is based on the environmental conditions that are necessary for the development of fungi. Some micromycetes, such as *Aspergillus*, are classified as storage fungi in temperate zones, but with the ability to infect grains in the field if there is an increase in temperature (Leggieri *et al*., 2020). Micromycetes become undesirable when they attack living organisms, either humans or animals, creating not only physiological and aesthetic discomfort but especially when they affect their health and endanger their lives (Taran *et al*., 2020).

Here are some of the reasons why micromycetes deserve to be studied because the microscopic world was born, evolved and perfected before man was defined as an all-knowing, all-encompassing and all-mastering being.

MATERIAL AND METHOD

The number of filamentous micromycetes in the vegetal substrates was determined based on the method of serial dilutions, according to the standard SR EN ISO 16140-2. After the macroscopic examination of the colonies, preparations were made between the blade and the lamella, being subsequently analyzed under a microscope with a 10x and 40x objective to highlight the species of fungi. The aim was to prevent and limit fungal contamination of plant substrates.

RESULTS AND DISCUSSIONS

Table 1 presents the results on the incidence and percentage expression of the main genera of dominant micromycetes inventoried and identified in 150 analyzed plant substrates.

Table 1

Selective results of qualitative mycological exam of the analyzed plant substrates

Sample	Nr. p.a.	Asp.		Pen.		Fus.		Clad.		Altern.		Mucor.	
		P.p.	%	P.p.	%	P.p.	%	P.p.	%	P.p.	%	P.p.	%
Alfalfa half-hay	30	4	13	9	30	4	13	4	13	2	6.6	3	10
Corn silo	30	15	50	8	26.6	-	-	-	-	8	26.6	6	20
Mixed feed ration	30	-	-	10	33.3	11	36.6	-	-	-	-	8	26.6
Concentrates	30	3	10	3	10	-	-	-	-	-	-	1	3.3
Corn grains	30	-	-	-	-	12	40	6	20	6	20	-	-
TOTAL	**150**	**22**	**73**	**30**	**99.9**	**27**	**89.6**	**10**	**33**	**16**	**53.2**	**18**	**59.9**
		Asp.		**Pen.**		**Fus.**		**Clad.**		**Altern.**		**Mucor.**	

No.p.- Number of samples analyzed, P.p.- Positive samples, Asp-Aspergillus, Pen-Penicillium, Fus-Fusarium, Clad-Cladosporium, Mucor-Mucoraceae

The corn silo showed the highest degree of contamination (50%) with species belonging to the genus *Aspergillus*, and in second place were the corn grains with a percentage of 40% with species of the genus *Fusarium*. In third place was the mixed feed ration, which offers optimal conditions for the multiplication of micromycetes belonging to the genera *Penicillium* (33.3%) and *Fusarium* (36.6%). The timing of corn harvesting is very important, depending on its variety. Also, the humidity must be between 25 - 27%. If corn is left in the field for a longer period of time, the risk of mycotoxin contamination increases considerably. Corn should dry up to 16% moisture in less than a day or two after harvest, as fungi of the genus *Penicillium, Fusarium* grow rapidly in moist corn (García-Díaz *et al*., 2020).

The interpretation with a higher objectivity of the obtained data can be highlighted by a graphical representation of the fungal genera, thus, it is counted that the genus *Penicillium* dominates the entire mycotic mosaic that characterizes the examined plant substrates, being present in 99.9% of the analyzed samples. The dominant fungal flora is the genus *Fusarium* with a participation rate of 89.6% and the genus *Aspergillus* (73%). It is noteworthy that on the 4th place are

the species from the *Mucoraceae* family, which occupy 59.9% in this hierarchy (fig. 1).

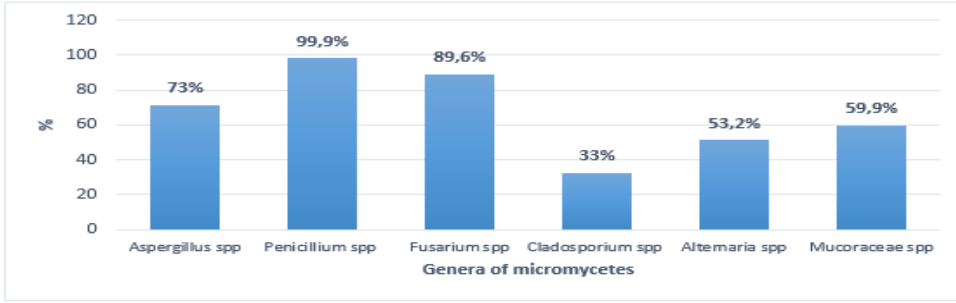

Fig. 1 Graphical representation of the genera of micromycetes isolated from the analyzed vegetal substrates

The genus *Penicillium* (30%) was identified in the alfalfa seed samples (fig. 2), being followed by the genera *Aspergillus*, *Fusarium* and *Cladosporium* with the same percentage of 13%, and in the corn silage and concentrates were found fungi of the genera *Aspergillus*, *Penicillium* and the *Mucoraceae* family. The genera *Alternaria*, *Cladosporium* and *Fusarium* were not present in the concentrate samples.

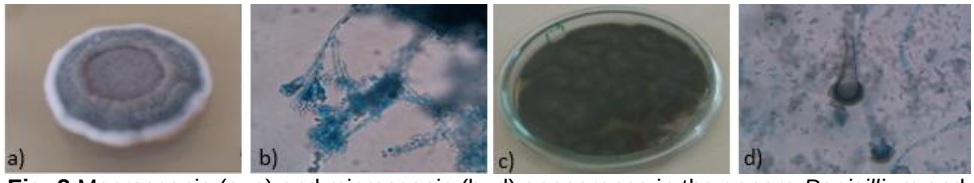

Fig. 2 Macroscopic (a, c) and microscopic (b, d) appearance in the genera *Penicillium* and *Aspergillus*

Colonies of the genus *Penicillium* with a fluffy appearance (fig. 2a) and with the woolly mass, abundantly branched they are the result of the multiplication of aerial hyphae and conidiophores that appear as their arms. The genus *Penicillium* is best represented among mesophilic fungi in temperate zones.

In *A. fumigatus* (fig. 2c), the microscopic aspects aim at the presence of hyaline hyphae, the conidiophores are short, have a smooth wall and open towards the apical bridge, where they form a hemispherical vesicle (fig. 2d). These *Aspergilus* species contaminate plant substrates at different stages of their cultivation, after harvesting, storage or processing.

The *Fusarium* strain grows well on solid culture media at a temperature of 25^{o}C, its vegetative apparatus consisting of fluffy colonies, which tend to occupy in a few days the entire available area (fig. 3).

Fig. 3 Macroscopic (a, b) and microscopic (c) appearance of the genus *Fusarium*

Fusarium fungi are contaminated elements that are frequently found in atmospheric air, behaving under certain conditions as opportunistic germs. It adapts to the most diverse pedoclimatic conditions, its species being found both in cold and humid soils and in warm areas. The fourth place was held by micromycetes from the broad category of mucoraceae that grow on substrates with higher humidity (18%). The fungi of the *Mucoraceae* family also have the highest resistance to dry heat.

In Romania, in the N-E areas, the species of the genus *Alternaria* -53.2% (fig. 4) are isolated in vegetal substrates with a frequency worthy of emphasis. Most of the species of the genus *Alternaria* are pathogenic to plants and only very rarely can some strains acquire the qualities of opportunistically conditioned germs pathogenic to humans and animals. The *Alternaria* colony alternates on the obverse is well outlined, the coloration is gray-gray, the conidial surface slightly fluffy, finely velvety, and on the reverse the surface of the conidia is furrowed or marked by obvious ditches (fig. 4a,b).

Fig. 4 Macroscopic (a, b) and microscopic (c) appearance of the genus *Alternaria*

Being less pretentious to the environmental conditions, the species of the genus *Cladosporium* were highlighted in 10 samples (33%), being dominated by the expansive capacity of *Mucoraceae* and species of the genus *Alternaria*. In figure 5, the macroscopic appearance of the genus *Cladosporium* shows colonies with a velvety texture, rarely powdery, depending on the age of the micromycete and the nature of the culture medium.

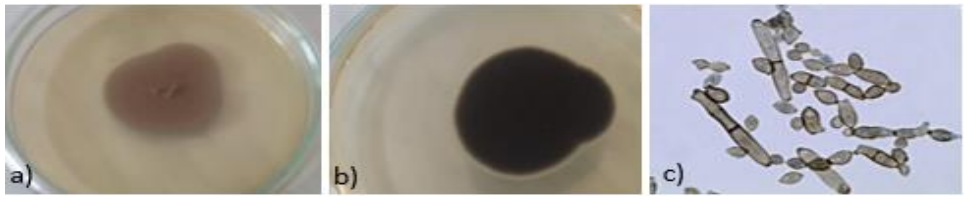

Fig. 5 Macroscopic (a, b) and microscopic (c) appearance of the genus *Cladosporium*

Both the obverse and the reverse of the colonies are pigmented, the conidial surface in shades ranging from dark olive-gray to blackish-brown on the PDA medium, and the reverse remains constant in darker and more uniform shades. *Cladosporium* species are micromycetes widespread in nature and are easily isolated from the analyzed plant substrates. Spores of this genus are also included in the category of aerogenic fungi.

CONCLUSIONS

1. The most contaminated samples were corn silage and corn grains with species belonging to the genera *Aspergillus* (50%) and *Fusarium* (40%), respectively. In N-E Romania, the species of fungi belonging to the genera *Penicillium* (99.9%), *Fusarium* (89.6%) and *Aspergillus* (73%) predominated, the spores of these genera being classified in the category of aerogenic fungal flora.

2. The climatic conditions in this geographical area are favorable for these species of micromycetes because in specialized laboratories, on environments specific to micromycetes, they develop a vigorous vegetative apparatus with a phenotypic expression difficult to confuse and easily taxonomic, if the data are corroborated and with the morphological and structural particularities of the fruiting bodies.

REFERENCES

1. **García-Díaz M., Gil-Serna J., Vázquez, C., Botia M.N., Patiño B.A., 2020 -** *A comprehensive study on the occurrence of mycotoxins and their producing fungi during the maize production cycle in Spain*. Microorganisms, Vol. 8, p. 141.
2. **Khodaei K., Rocha L., Savi G., Carnielli-Queiroz L., De Carvalho Fontes L., Correa B., 2019 -** *Assessment of toxigenic fusarium species and their mycotoxins in brewing barley grains*. Toxins, Vol. 11 (31).
3. **Leggieri M.C., Lanubile A., Dall'Asta C., Pietri A., Battilani P., 2020 -** *The impact of seasonal weather variation on mycotoxins: maize crop in 2014 in northern Italy as a case study*. World Mycotoxin Journal. Vol. 13 (1), p. 25-36.
4. **Mohale S., Medina A., Rodríguez A., Sulyok M., Magan N., 2013 -** *Mycotoxigenic fungi and mycotoxins associated with stored maize from different regions of Lesotho*. Mycotoxin Res. Vol. 29, p. 209–219.
5. **Munkvold G.P., 2017 -** *Fusarium species and their associated mycotoxins*. In Mycotoxigenic Fungi: Methods and Protocols, p. 51–106.
6. **Oldenburg E., Höppner F., Ellner F., Weinert J., 2017 -** *Fusarium diseases of maize associated with mycotoxin contamination of agricultural products intended to be used for food and feed.* Mycotoxin Research. Vol. 33 (3), p. 167–182.
7. **Pfordt A., Romero L.R., Schiwek S., Karlovsky P., Tiedemann A., 2020 -** *Impact of environmental conditions and agronomic practices on the prevalence of Fusarium species associated with ear- and stalk rot in maize.* Pathogens, Vol. 9, p. 236.
8. **SR EN ISO 6887-1:2017 -** *Microbiology of the food chain - Preparation of test samples for microbiological examination - General rules the preparation of initial suspension and decimal dilutions*
9. **Taran G.V., Pugach S.G., Zamuriev A.A., Opalev P.O., Yaroshenko M.O., 2020 -** *Plasma-chemical method of grain fungal contamination control.* Problems of atomic science and technology. Vol. 6, p. 127-130.