ASSESSMENT OF THE ACTION OF DEPOSIT MYCOFLORA ON *TRITICUM AESTIVUM* L. SEEDS FROM SUCEAVA GENEBANK'S COLLECTION

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Keywords: micromycetes, CGA medium, blotting paper
Abstract: This study consisted in a phytopathological evaluation of epiphyte and endophyte mycological flora which appeared on Triticum aestivum seeds placed on two types of substrates (CGA medium and blotting paper). The 30 populations of wheat resulted from the active collection of Suceava Genebank and conserved for different time intervals (8, 15 and 18 years), in controlled atmosphere storages (T=+4^{0}C; relative air humidity = 30 - 40%). Micromycetes were evaluated by counting the infected seeds and the attack frequency was expressed as a percentage, by visual estimation of seeds surface. The target objectives of the study are to establish the influence of the conservation period on the activity of micromycetes placed on stored seeds and to settle the influence of the substrate type - CGA medium (potato - dextrose - agar) and blotting paper - on the development of fungal pathogens. Seeds studied, placed on CGA medium and blotting paper substrate, after incubation, showed a different degree of infection by fungal pathogens, depending on the type of substrate and the age of seeds. The conservation period influenced fungal pathogens longevity, meaning that the more it's higher, the level of infection is reduced. On CGA medium, in comparison with blotting paper substrate, after incubation period, was isolated a greater diversity of fungal pathogens.

INTRODUCTION

Size variation of pathogen colonies kept in constant environmental conditions reflects the differences in quantity, viability and location of inoculums on seed. The development of colony around each seed on growth medium, and the intensity of symptoms on germs in case of blotting paper tests are closely dependent on the amount of inoculum on seed - number of spores or mycelium abundance (Raicu and Baciu, 1978).

Generally, the correlation between inoculum (spores load/seed) and colony size (the amount of mycelium) developed on CGA medium (potato - dextrose - agar) is very significant (Hulea et al. 1973).

Micromycetes existing on stored wheat seeds can cause during storage a wide range of changes, with negative consequences from a technological, nutritional, hygienic and commercial point of view (Nagy and Trif, 1998).

Beratlief and collaborators in a study concerning the deposit ecosystem characteristics, revealed the mycological flora evolution and sequence on cereals seeds stored with high moisture content (Beratlief and Oprea, 1994)

The purposes of this study are:

- to establish the influence of the conservation period on the activity of micromycetes placed on stored seeds
- to settle the influence of the substrate type - CGA medium (potato - dextrose - agar) and blotting paper - on the development of fungal pathogens.
- to establish the complementary action of identified micromycets on *Triticum aestivum* seeds in three storage periods, by determining the correlation coefficients between the action of fungal pathogens identified on the samples taken in study.

MATERIALS AND METHODS

I performed the phytopathological characterization of local germplasm represented by 30 populations of *Triticum aestivum,* conserved for 8, 15 and 18 years at T = +4°C, which come from collecting expeditions realized by the collecting department from Suceava Genebank during a term of 18 years (1992-2010).

Lab experiments were carried on Suceava Genebank by using the genetic seminal material from the active collection of the institution, which was placed on the CGA medium and blotting paper.

To make possible the assessment of the micromycetes present on *Triticum aestivum* seeds, I implemented the following research methods:

- macroscopic analyses of the seeds;
- Ulster method (Malone and Muskett, 1941) on CGA medium (potato - dextrose - agar).

Interpretation of results concerning identified micromycetes evolutions on seeds taken in study was achieved by analyzing correlations and regressions accordingly with experimental factors (Ceapoiu, 1968).

RESULTS AND DISCUSSIONS

The seeds of *Triticum aestivum,* placed on CGA medium and blotting paper, presented after the incubation period the following characteristics concerning the presence of fungal microorganisms:

a) CGA medium (potato - dextrose - agar)

On CGA medium, the presence of deposit mycoflora on the 30 samples of *Triticum aestivum* seeds conserved at $+4^0$C temperature, for 8, 15 and 18 years was different, as follows:

On the samples stored for 8 years at $+4^0$C temperature, we identified 9 fungal pathogens (*Penicillium sp, Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Trichoderma viride, Torula herbarum, Stemphylium botryosum, Chaetomium sp.)* which showed a different attack degree on each sample of the 5 analyzed, registering an infection rate of 97,3%.

On 21 samples stored at $+4^0$C temperature for a period of 15 years we identified 8 fungal pathogens (*Penicillium sp., Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Trichoderma viride, Stemphylium botryosum, Chaetomium sp.*). The 630 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 33,3%.

Other 4 seed samples. conserved for a period of 18 years, have been infected by a smaller number of fungal microorganisms (*Penicillium sp., Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata)* and the infection percentage on the 120 seeds analyzed was much lower (35 %).

In table 1 are presented the micromycetes identified on *Triticum aestivum* seeds placed in three experimental conditions (8, 15 and 18 years) at $+4^0$C temperature:

Table 1. Proportion of micromycetes isolated on *Triticum aestivum* seeds placed on CGA medium

Experimental conditions	**Seeds stored at T + 4^0C, for 8 years**	**Seeds stored at T+ 4^0C, for 15 years**	**Seeds stored at T+4^0C, for 18 years**
Isolated micromycets	**Attack frequency (%)**		
Penicillium sp.	18,6	9,2	3,3
Rhizopus sp.	21,3	8,9	13,3
Epicoccum sp	5,3	2,8	4,2
Cladosporium herbarum	7,3	3,3	4,2
Alternaria alternata	15,3	6,2	10
Trichoderma viride	11.4	0,9	0
Torula herbarum	6,7	0	0
Stemphylium botryosum	12,7	0,6	0
Chaetomium sp.	3,3	1,3	0
TOTAL	**97,2**	**33,2**	**40.2**

Proportion of micromycets isolated on 30 seeds samples of *Triticum aestivum* placed on CGA medium stored at + 4 ^{0}C temperature for 8, 15 and 18 years is represented in figure 1:

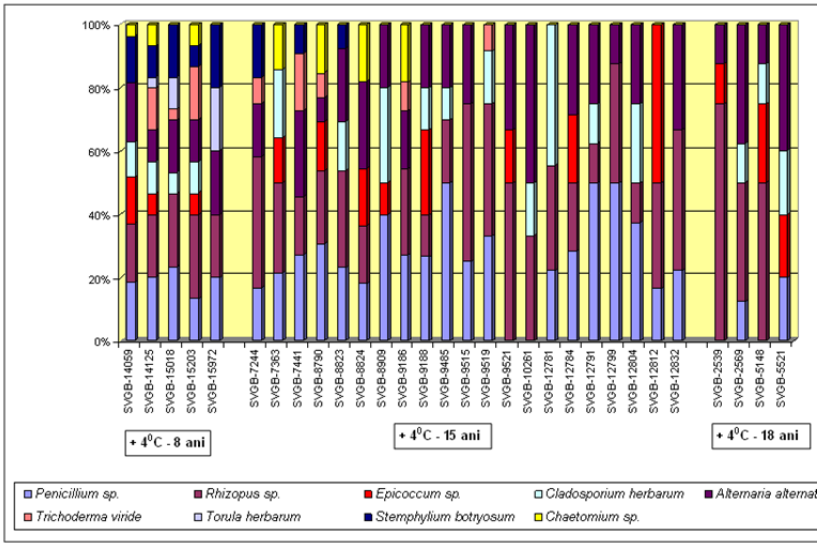

Fig.1. Infection percentages of fungal pathogens isolated on *Triticum aestivum* seeds placed on CGA medium in controlled atmosphere conditions

b) blotting paper

Analyzing the 30 seeds samples of *Triticum aestivum* stored at +4^{0}C temperature for 8, 15 and 18 years, we identified the following infection percentages caused by fungal pathogens:

On the samples stored for 8 years at + 4^{0}C temperature we have identified 6 fungal pathogens (*Penicillium sp., Rhizopus sp., Cladosporium herbarum, Alternaria alternata, Trichoderma viride, Torula herbarum*), which had a different attack degree on each sample of the 5 analyzed, registering an infection rate of 41,6% (table 2).

Table 2. Proportion of micromycetes isolated on *Triticum aestivum* seeds placed on blotting paper

Experimental conditions	**Seeds stored at T +4^{0}C, for 8 years**	**Seeds stored at T + 4^{0}C, for 15 years**	**Seeds stored at T+ 4^{0}C, for 18 years**
Isolated micromycets	**Attack frequency (%)**		
Penicillium sp.	8	3,5	1
Rhizopus sp.	22,4	5,5	4
Cladosporium herbarum	2,8	0,6	0,5

Experimental conditions	Seeds stored at T +4^{0}C, for 8 years	Seeds stored at T + 4^{0}C, for 15 years	Seeds stored at T+ 4^{0}C, for 18 years
Alternaria alternata	3,6	2	1,5
Trichoderma viride	2	0,6	0
Torula herbarum	2,8	0	0
TOTAL	**41,6**	**12,2**	**7**

On 21 samples conserved at +4^{0}C temperature for a period of 15 years, we identified 5 fungal pathogens **(***Penicillium sp., Rhizopus sp., Cladosporium herbarum., Alternaria alternata., Trichoderma viride)*. The 1050 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 12,19 %.

The 4 seed samples with a storage period of 18 years have been infected by a smaller number of micromycetes (*Penicillium sp., Rhizopus sp., Cladosporium herbarum, Alternaria alternata),* the infection percentage on the 200 seeds analyzed being more low (7 %).

Analyzing the number of infected seeds, we can observe that all micromycetes genus of wheat seeds were isolated on a smaller number of seeds when samples were incubated on blotting paper, in comparison with the number of seeds placed on CGA medium.

Proportion of micromycets isolated on 30 seeds samples of *Triticum aestivum* placed on blotting paper stored at + 4 ^{0}C temperature for 8, 15 and 18 years is represented in figure 2.

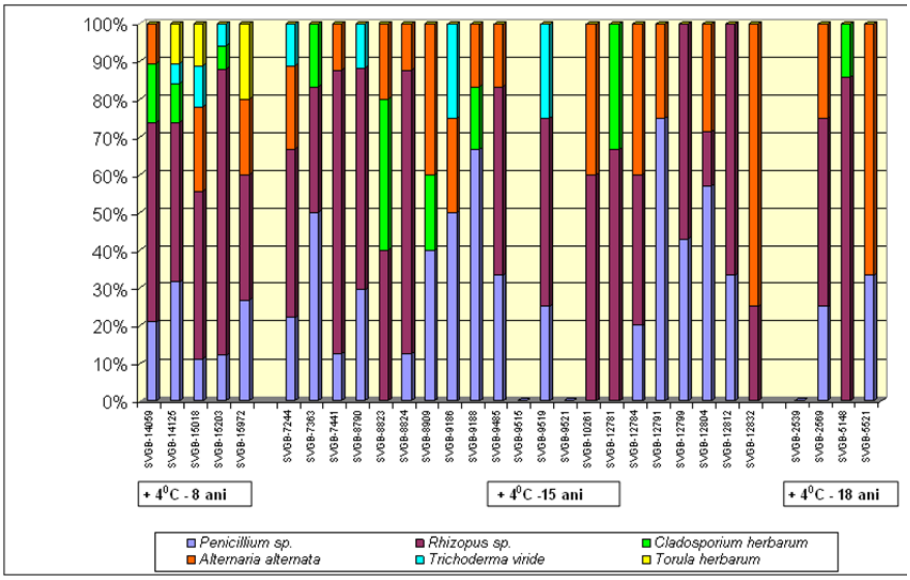

Fig.2.Infection percentages of fungal pathogens isolated on *Triticum aestivum* seeds placed on blotting paper in controlled atmosphere conditions

For establishment complementary action of micromycets identified on *Triticum aestivum* seeds in three storage periods (8, 15 and 18 years), it was determined correlation coefficients between fungal pathogens action identified on samples taken in study.

In the analyzed samples of *Triticum aestivum*, the results from the table related few statistical correlations in three storage periods.

After 8 years of storage of *Triticum aestivum* seeds at +4°C temperature (table 3), it's noticed that there is few significant positive correlations between micromycetes action: *Stemphylium botryosum x Torula herbarum, Stemphylium botryosum x Alternaria alternata, Chaetomium sp. x Trichoderma viride.*

Table 3. Correlation coefficients between micromycetes action identified on *Triticum aestivum* samples stored at +4°C, for 8 years

Caracterele corelate	*Penicillium sp.*	*Rhizopus sp.*	*Epicoccum sp.*	*Cladosporium herbarum*	*Alternaria alternata*	*Trichoderma viride*	*Torula herbarum*	*Stemphylium botryosum*	*Chaetomium sp.*
Penicillium sp.	1								
Rhizopus sp	-0,23077	1							
Epicoccum sp.	-0,62897	-0,4193	1						
Cladosporium herbarum	-0,43724	0,10090	0,73335	1					
Alternaria alternata	0,23076	-0,2307	-0,3669	-0,7735	1				
Trichoderma viride	-0,46747	0,65446	0,12741	0,57231	**-0,84145***	1			
Torula herbarum	0,60201	-0,086	**-0,8204***	**-0,977*****	0,688021	-0,543	1		
Stemphylium botryosum	0,69337	-0,4160	-0,5669	**-0,8488***	**0,83205***	**-0,87600***	**0,8682***	1	
Chaetomium sp.	-0,65779	0,21926	0,59761	0,76696	**-0,87706***	**0,85280***	-0,7844	**-0,9486****	1

After 15 years of storage of *Triticum aestivum* seeds at +4°C temperature, there is only one very significant positive correlation between fungal pathogens action *Stemphylium botryosum x Trichoderma viride* (table 4).

Table 4. Correlation coefficients between micromycetes action identified on *Triticum aestivum* samples stored at +4°C temperature, for 15 years

Caracterele corelate	*Penicillium sp.*	*Rhizopus sp.*	*Epicoccum sp.*	*Cladosporium herbarum*	*Alternaria alternata*	*Trichoderma viride*	*Stemphylium botryosum*	*Chaetomium sp.*
Penicillium sp.	1							
Rhizopus sp.	-0,07395	1						
Epicoccum sp.	0,091247	-0,12263	1					
Cladosporium herbarum	0,224141	-0,09279	-0,03097	1				
Alternaria alternata	0,038123	-0,1964	0,084293	-0,33072	1			
Trichoderma viride	0,153525	0,279145	-0,21965	-0,28204	-0,01066	1		
Stemphylium botryosum	-0,07255	0,407718	-0,26249	-0,15448	0,210195	**0,49793***	1	
Chaetomium sp.	0,083883	0,129641	0,250387	-0,09824	-0,14852	0,18999	-0,185	1

Therefore, after 18 years of conservation there is only one very significant positive correlation between fungal pathogens action *Cladosporium herbarum x Penicillium sp.* (table 5).

Table 5. Correlation coefficients between micromycetes action identified on *Triticum aestivum* samples stored at +4°C temperature, for 18 years

Caracterele corelate	*Penicillium sp.*	*Rhizopus sp.*	*Epicoccum sp.*	*Cladosporium herbarum*
Penicillium	1			

Caracterele corelate	*Penicillium sp.*	*Rhizopus sp.*	*Epicoccum sp.*	*Cladosporium herbarum*
sp.				
Rhizopus sp.	-0,40825	1		
Epicoccum sp.	-0,30151	-0,73855	1	
Cladosporium herbarum	**0,904534***	-0,49237	-0,09091	1

Also, it is observed a constant presence of fungal pathogen C*ladosporium herbarum.*

For setting of the two micromycetes action (*Cladosporium herbarum x Torula herbarum)* present on seeds after 8 years of storage, it was traced the suitable regression straight (fig. 3).

This regression straight line out negative significant action of the two saprophytic micromycetes, meaning that while maintaining seeds at +4^{0}C temperature, after 8 years of storage, *Torula herbarum* inhibate action of fungal pathogen *Cladosporium herbarum.*

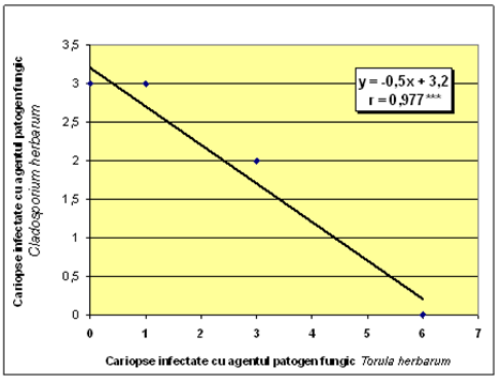

Fig. 3. The regression straight for correlation between number of infected seeds of *Torula herbarum* and number of infected seeds of *Cladosporium herbarum* on *Triticum aestivum* samples stored in controlled environmental conditions (+ 4^{0}C) for 8 years

CONCLUSIONS

Deposit mycoflora developed on wheat seeds taken in this study was analyzed according to genotype period of seed conservation and type of substrate used.

The seed samples of *Triticum aestivum* stored in 3 experimental conditions placed on CGA medium were infected in different proportions by fungal pathogens. The species *Torula herbarum* was identified only on the samples conserved for 8 years at +4^{0}C temperature and the species *Trichoderma viride, Stemphylium botryosum*, *Chaetomium sp.* were identified only on the

seeds with a storage period of 8 and 15 years. Other types of micromycetes were detected in all storage conditions, but on a different number of seeds (*Penicillium sp, Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata).*

By placing the same seed samples of *Triticum aestivum* in 3 experimental conditions on blotting paper, we observed that samples were infected in a smaller proportion compared to CGA medium. The fungal pathogens *Epicoccum sp., Stemphylium botryosum*, *Chaetomium sp.* identified on CGA medium, were not isolated on blotting paper.

After 8 and 18 years of storage of *Triticum aestivum* seeds at +4°C temperature, there is a strong attack of *Rhizopus sp.* and *Alternaria alternata,* being a very significant correlation between the action of these two fungal pathogens. *Epicoccum sp.*was identified on a great number of samples, but the infection degree was observed in a small number of seeds.

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