THE INFLUENCE OF THE CONSERVATION PERIOD ON THE ACTIVITY OF MYCOLOGICAL FLORA ON *ZEA MAYS* SEEDS FROM SUCEAVA GENEBANK'S COLLECTION

DIANA BATÎR – RUSU[1*]

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Abstract: The purposes of the study were 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.

This study consisted in a phytopathological evaluation of epiphyte and endophyte mycological flora which appeared on *Zea mays* seeds placed on two types of substrates (CGA medium and blotting paper). The 30 populations of corn resulted from the active collection of Suceava Genebank and conserved for different time intervals (8 and 17 years), in controlled atmosphere storages (T=+4^{0}C; relative air humidity = 30 - 40%).

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.

Micromycetes were evaluated by counting the infected seeds and the attack frequency was expressed as a percentage, by visual estimation of seeds surface.

The conservation period influenced fungal pathogens longevity, meaning that the more it's higher, the level of infection is reduced.

On CGA medium, compared with blotting paper substrate, after incubation period, was isolated a greater diversity of fungal pathogens.

The experimental results of this study answered the following objectives:

- identification of fungal microorganisms according to storage period of seeds;
- identification of fungal genera depending on the type of substrate used;
- setting of correlations between micromycetes identified evolution, seed storage periods and the type of substrate used.

INTRODUCTION

The transmission of fungal pathogens by seeds has always been the most rapidly spreading of diseases from one region to another and therefore it's need to know the symptoms, the biology and mode of transmission of pathogens, for once reported to be ensured effectively preventing and fighting them (Baker K.F., 1966).

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.

Viability of inoculum seed - borne is in some cases directly related to interspace, harvesting - storage - analysis. Some fungal pathogens are present and can be found in high percentages in freshly harvested seeds, but their infection it's reducing significantly after one year of storage (Hulea, A., Negru, Al., Severin, V. 1973).

Micromycetes existing on stored cereals seeds can cause during storage a wide range of changes, with negative consequences from a technological, nutritional, hygienic and commercial point of view (Nagy, E., Trif, V., 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, C., Oprea, M**.** 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 micromycetes on *Zea mays* seeds in two storage periods, by determining the correlation coefficients between the action of fungal pathogens identified on the samples taken in study.

MATERIALS AND METHODS

It was performed the phytopathological characterization of local germplasm represented by 30 populations of *Zea mays,* conserved for 8 and 17 years at T = +4°C, which come from collecting expeditions realized by the collecting department from Suceava Genebank during a term of 10 years (1993-2010).

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 *Zea mays* seeds, It was implemented the following research methods :

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

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

RESULTS AND DISCUSSIONS

The seeds of *Zea mays,* 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 *Zea mays* seeds conserved at +4 ^{0}C temperature, for 8 and 17 years was different, as follows:

On the samples stored for 8 years at +4 ^{0}C temperature, we identified 9 fungal pathogens (*Penicillium sp., Aspergillus sp.*, *Rhizopus sp., Mucor sp*., *Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Fusarium moniliforme, Oedocephalum sp.*) which showed a different attack degree on each sample of the 5 analyzed, registering an infection rate of 75 % (112 infected seeds of 150 analyzed) (table 1).

On 25 samples stored at +4^{0}C temperature, for a period of 17 years we identified 9 fungal pathogens (*Penicillium sp., Aspergillus sp.*, *Rhizopus sp., Mucor sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Fusarium moniliforme, Chaetomium sp.*). The 750 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 59 %, being infected 443 seeds. In these storage conditions, the species *Oedocephalum sp.* not expressed at all.

Table 1. Proportion of micromycets isolated on *Zea mays* seeds placed on CGA medium

Experimental conditions	**Seeds stored at T + 4^{0}C, for 8 years**	**Seeds stored at T + 4^{0}C, for 17 years**
Isolated micromycets	**Attack frequency (%)**	
Penicillium sp.	32	20,5
Aspergillus sp.	4	3,1
Rhizopus sp.	11,3	23,2
Mucor sp.	4	2

Experimental conditions	Seeds stored at T + 4^0C, for 8 years	Seeds stored at T + 4^0C, for 17 years
Cladosporium herbarium	2	1,5
Alternaria alternata	2	1,5
Trichothecium roseum	2	1,1
Chaetomium sp.	0	1,5
Fusarium moniliforme	14,6	4,8
Oedocephalum sp.	2,6	0
TOTAL	**74,5**	**59,2**

Proportion of micromycets isolated on 30 seeds samples of *Zea mays* placed on CGA medium stored at + 4 ^{0}C temperature for 8 and 17 years is represented in figure 1:

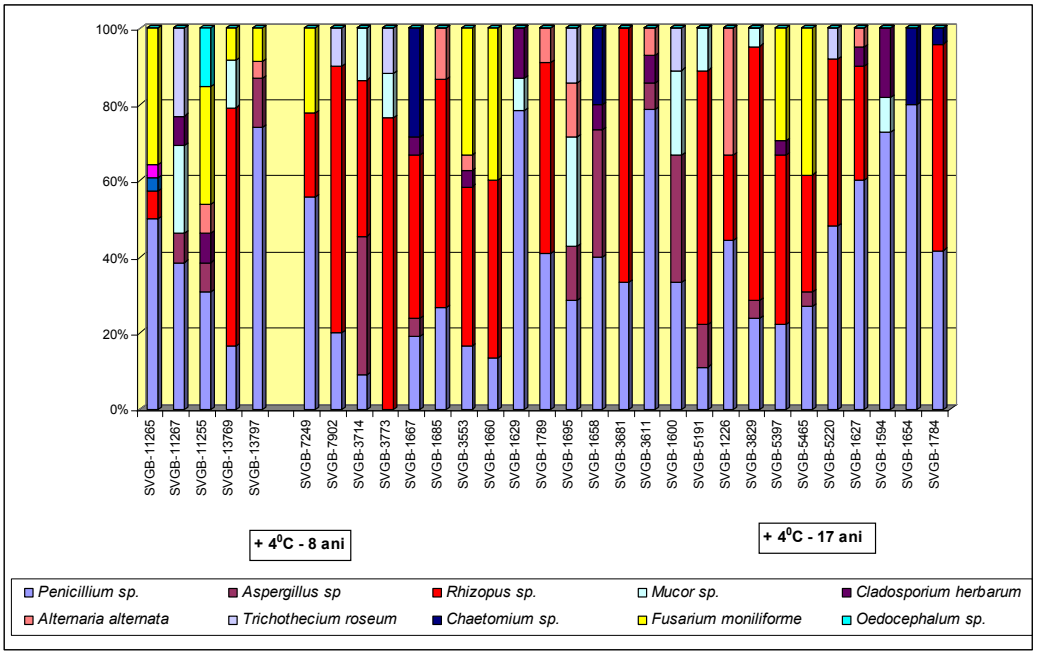

Fig.1. Infection percentages of fungal pathogens isolated on *Zea mays* seeds placed on CGA medium in controlled atmosphere conditions

b) blotting paper

For emphasing the role of substrate used for analysis of micromycetes occuring on *Zea mays* seeds after different periods of storage, we used also blotting paper substrate.

Analyzing the 30 seed samples of *Zea mays* stored at +4^{0}C temperature for 8 and 17 years it was identified the following infection percentages caused by fungal pathogens.

On the samples stored for 8 years at +4^{0}C temperature was identified 5 fungal pathogens (*Penicillium sp,. Rhizopus sp., Aspergillus sp., Cladosporium herbarum, Alternaria alternata,*)

which had a different attack degree on each sample of the 5 analyzed registering an infection rate of 20,4 % (table 2).

On 25 samples conserved at +4^{0}C temperature for a period of 17 years it was identified 6 fungal pathogens (*Penicillium sp., Aspergillus sp., Rhizopus sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum)*. The 1250 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 9,12 %.

Table 2. Proportion of micromycets isolated on *Zea mays* 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 17 years**
Isolated micromycets	**Attack frequency (%)**	
Penicillium sp.	8,4	2,8
Aspergillus sp.	2,4	0,7
Rhizopus sp.	3,2	3,6
Cladosporium herbarium	2	0,9
Alternaria alternata	4,4	0,7
Trichothecium roseum	0	0,4
TOTAL	**20,4**	**9,1**

Proportion of micromycets isolated on 30 seeds samples of *Zea mays* placed on blotting paper stored at + 4 ^{0}C temperature for 8 and 17 years is represented in figure 2:

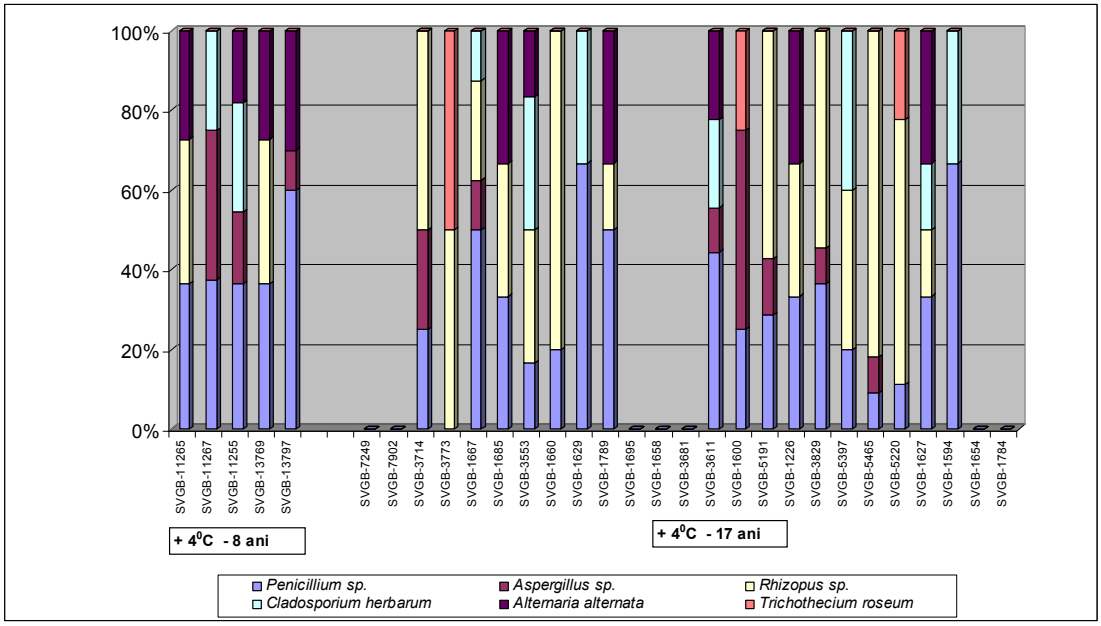

Fig.2. Infection percentages of fungal pathogens isolated on *Zea mays* seeds placed on blotting paper in controlled atmosphere conditions

For establishment complementary action of micromycetes identified on *Zea mays* seeds in two storage periods (8 and 17 years), it was determined correlation coefficients between fungal pathogens action identified on samples taken in study.

In the analyzed samples of *Zea mays*, the results from the table related a lower number of statistical correlations in both storage periods.

After 8 years of storage of *Zea mays* seeds at +4°C temperature, it’s noticed that there is no one significant positive correlation between micromycetes action (table 3).

Table 3. Correlation coefficients between micromycets action identified on *Zea mays* samples stored at +4°C, for 8 years

Caracterele corelate	*Penicillium sp.*	*Aspergillus sp.*	*Rhizopus sp.*	*Mucor sp.*	*Cladosporium herbarum*	*Alternaria alternata*	*Trichothecium roseum*	*Fusarium moniliiforme*	*Oedocephalum sp.*
Penicillium sp.	1								
Aspergillus sp.	**0.485822**	**1**							
Rhizopus sp.	**-0.50563**	**-0.59789**	**1**						
Mucor sp.	**-0.81918**	**-0.4901**	**0.572099**	**1**					
Cladosporium herbarum	**-0.38361**	**0.300123**	**-0.43579**	**-0.10206**	**1**				
Alternaria alternata	**0.206558**	**0.728869**	**-0.43579**	**-0.61237**	**0.6875**	**1**			
Trichothecium roseum	**-0.45246**	**-0.08575**	**-0.29052**	**0.612372**	**0.25**	**-0.375**	**1**		
Fusarium moniliiforme	**0.353052**	**-0.19458**	**-0.21857**	**-0.71583**	**0.180499**	**0.309426**	**-0.56728**	**1**	
Oedocephalum sp.	**-0.15738**	**0.342997**	**-0.29052**	**-0.40825**	**0.875**	**0.875**	**-0.25**	**0.46414**	**1**

After 17 years of storage of *Zea mays* seeds at +4°C temperature, there is only two significant positive correlation between fungal pathogens action *Mucor sp. x Aspergillus sp.* and *Cladosporium herbarum x Penicillium sp.* (table 4).

Table 4. Correlation coefficients between micromycets action identified on *Zea mays* samples stored at +4°C temperature, for 17 years

Caracterele corelate	*Penicillium sp.*	*Aspergillus sp.*	*Rhizopus sp.*	*Mucor sp.*	*Cladosporium herbarum*	*Alternaria alternata*	*Trichothecium roseum*	*Chaetomium sp.*	*Fusarium moniliforme*
Penicillium sp.	**1**								
Aspergillus sp.	**-0.28225**	**1**							
Rhizopus sp.	**-0.22519**	**-0.13888**	**1**						
Mucor sp.	**-0.24304**	**0.53386***	**-0.11112**	**1**					
Cladosporium herbarum	**0.58568***	**-0.08949**	**-0.40892**	**0.07932**	**1**				
Alternaria alternata	**-0.00921**	**-0.21802**	**-0.14088**	**-0.24396**	**-0.12164**	**1**			
Trichothecium roseum	**-0.17687**	**-0.10722**	**0.233681**	**0.201709**	**-0.27657**	**-0.18534**	**1**		
Chaetomium sp.	**-0.08823**	**0.180856**	**-0.05727**	**-0.21671**	**0.17026**	**-0.18543**	**-0.16028**	**1**	
Fusarium moniliforme	**-0.06373**	**-0.16521**	**0.163364**	**-0.30488**	**0.002815**	**-0.12911**	**-0.22549**	**-0.1615**	**1**

CONCLUSIONS

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

From this study resulted the following conclusions :

The seeds samples of *Zea mays* stored in 2 experimental conditions placed on CGA medium, were infected in different proportions by fungal pathogens. The species *Oedocephalum sp.* was identified only on the samples conserved for 8 years at + 4^0C temperature and *Chaetomium sp.* was detected only on the samples conserved for 17 years.

By placing the same seed samples of *Zea mays* in 2 experimental conditions on blotting paper, we observed that samples were infected in a smaller proportion compared to CGA medium. The fungal pathogens *Mucor sp., Fusarium moniliforme, Oedocephalum sp., Chaetomium sp.* identified on CGA medium were not isolated on blotting paper.

Zea mays seeds stored for 17 years at +4°C temperature presented an additional infections with the species *Chaetomium sp*.

In all storage conditions, after 8 and 17 years storage of *Zea mays* seeds at +4°C temperature, there is a strong attack of *Penicillium sp., Rhizopus sp, Aspergillus sp.*

The fungal pathogens *Alternaria alternata* and *Cladosporium herbarum* were detected in

all storage conditions, on a lower number of samples and also the infection degree was observed in a small number of seeds.

After 17 years of storage of *Zea mays* seeds at +4°C temperature, there are two strong attack between *Mucor sp. x Aspergillus sp.* and also between *Cladosporium herbarum x Penicillium sp*. being a very significant correlation between the action of these fungal pathogens.

REFERENCES

Baker K.F., (1966). *Dynamics of seed transmission of plant pathogens*. Ann. Rev. Phytopathol.. 4. 311

Beratlief, C., Oprea, M. (1994), *Ecosystem characteristics of agricultural products storages and health implication,* Plant Protection issues, Bucharest, 13, 22, 44

Ceapoiu, N., (1968), *Statistical methods applied in agricultural and biological experiments,* Agro-Forestry Publishing, Bucharest

Hulea, A., Negru, Al., Severin, V. (1973), *Main Seed Crop Diseases,* Ceres Publishing, Bucharest, 35-70

Malone, J.P., Muskett, A.E., (1941), *The Ulster method for the examination of flax seed for the presence of seed borne parasites,* Annals Appl. Biol.. 8-13, 28

Nagy, E., Trif, V., (1998), *Research regarding prevention and control of seed borne diseases at cereals in terms of Transylvania,* Plant Protection issues, Bucharest, 26, 48-53

[1] Banca de Resurse Genetice Vegetale Suceava
* dia_sv@yahoo.com