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MICOFLORA ASSOCIATED WITH WHEAT SEEDS

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Abstract

The research followed the identification in laboratory conditions of the micoflora associated with wheat seeds. The biological material was represented by caryopsis from the Glosa and Boema varieties, used in the experiments monitored in our research. For both varieties, we worked with the PDA and malt agar culture medium variants and untreated seeds, seeds disinfected with distilled water and 70% ethanol solution. Each variant was represented in three repetitions. In the case of the Glosa variety, the most common micromycetes belonged to the genera Alternaria and Stemphylium, and in the case of the Boema variety, micromycetes from the genera Penicillium and Alternaria were detected. In both varieties, in the untreated(control) and disinfected with distilled water variants, Rhizopus spp. was established. The poorest micoflora was detected in the variant disinfecting the seeds with 70% ethanol solution. Seed germination was not affected.

Key words*: wheat, mycoflora, seeds, varieties.*

INTRODUCTION

Wheat is considered the most important cultivated plant, providing the basic food of people (Muntean et al., 2003) with an area that includes temperate and subtropical zones of the world (Hashmi & Ghaffar, 2006). Seeds are a common means of spreading and transmitting plant pathogens. Contaminated or infected seeds can contribute to the spread of some dangerous pathogens for plants with the establishment of crops but also in the case of commercial exchanges (Agarwal & Sinclair, 1996). Seeds from diseased plants can cause reduction or inhibition of germination, plant growth, yield reduction and crop quality deterioration (Webwer et al., 2001; Dawson & Bateman, 2001; Bateman & Kwasna, 1999). The pathogens present internally or contaminating the seeds or associated in the seed mass are sources for the infections of cultivated plants.

The micoflora of wheat seeds transmitted through wheat seeds contributes to the quantitative and qualitative decrease of wheat production. The micoflora of wheat seeds is associated with pathogens such as: *Fusarium* spp., *Alternaria* spp., *Drechslkera sorokiniana*, *Cladosporium herbarum*, *Stemphylium botryosum* (Glazek, 1997; Nirenberg et al., 1994). Also, micromycetes from the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Trichoderma, Trichotecium*, *Tilletia* are associated with the micoflora of wheat seeds (Hashmi & Ghaffar, 2006; Raicu & Baciu, 1978; Hajihasani et al., 2012).

Micromycetes associated with wheat seeds can, under certain conditions, cause black point, a condition of the seeds that can negatively influence germination (Cristea et al. 2008; Gheorghieş et al., 2004). Also important is the fact that they are fungi that contaminate seeds and are sources of some mycotoxins (Chulze, 2010; Ittu M., 2006) dangerous for consumers in the food chain.

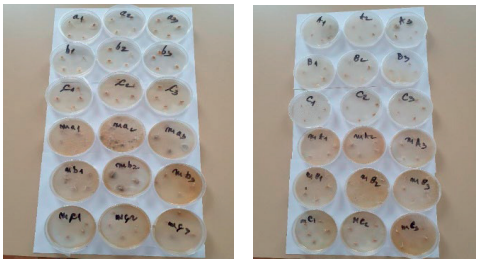
MATERIALS AND METHODS

The research aimed to identify the mycoflora of wheat seeds. The biological material was represented by seeds from the Glosa and Boema varieties, used in experiments set up during the research period at Moara Domneasca. The variants analyzed were not

disinfected, disinfected in distilled water and disinfected in 70% ethanol solution. Three repetitions were made for each variant. Petri dishes with a diameter of 90 mm and the culture medium PDA Roth (potato extract glucose agar) intended for microbiology laboratories and malt agar were used. Samples of 15 seeds/variant were made. The disinfected seeds were placed in Petri dishes incubated at a thermostat at 21^0C±20^0C. The mycelial growth was monitored 3, 6, 9 days. After the incubation period each Petri dish was examined and the microscopic identification of the pathogens present. The incidence of seeds infected with the identified pathogen was calculated as the percentage of seed infection of each variant, with the formula: F (I) = (n/N) x 100, where, F(I) = frequency (incidence) of seed infection; n= number of seeds on which a fungal species was identified; N = number of seeds tested. The presence of the micromycete *Rhizopus* spp was measured as a percentage of the surface of the Petri plates. The microscopic identification was carried out according to the morphological characteristics of the fructifications of the fungi.

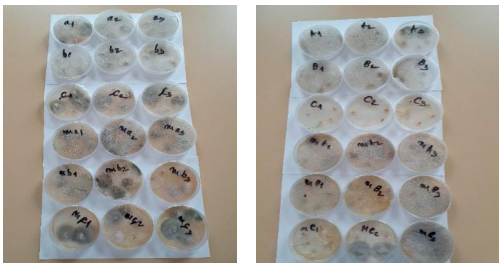
RESULTS AND DISCUSSIONS

For the examination of the micoflora, seeds from the varieties Glosa (A) and Boema (B) placed on PDA and MA culture media were used. The vegetative growth of the fungi was monitored after 3 days (Figure 1), after 6 days (Figure 2) and after 9 days (Figure 3) of incubation. After 9 days of incubation, the microscopic identification of the pathogens identified according to the characteristics of the specific fruits was carried out.



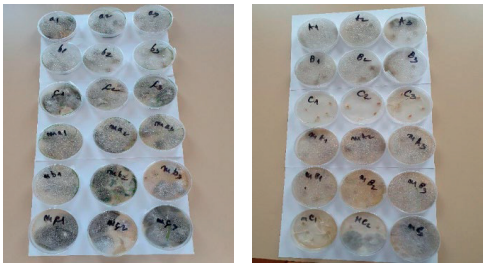
A (Glosa) B (Boema)

Figure 1. Growth of fungal colonies on PDA (A) and MA (B) culture medium 3 days after incubation



A (Glosa) B (Boema)

Figure 2. Growth of fungal colonies on PDA (A) and MA (B) culture medium 6 days after incubation



A (Glosa) B (Boema)

Figure 3. Growth of fungal colonies on PDA (A) and MA (B) culture medium 9 days after incubation

The data in Table 1 show the microflora associated with wheat seeds in the monitored varieties. In the case of the Glosa variety, on the PDA culture medium in the control variant, the micromycetes *Alternaria* spp. and *Stemphylium* spp. were detected in all the vessels, and in two of the repetitions the pathogen *Epiccocum* spp was also detected. In the distilled water variant, *Penicillium* spp. and *Alternaria* spp. and *Stemphylium* spp. were detected.

In the case of the 70% ethanol solution variant, fruiting belonging to the genera *Alternaria* and *Stemphylium* were identified, with poor fruiting. Regarding the development of the colonies and the identification of fungi on the malt agar medium, a richer fungal flora was found, possibly because the light disinfection with distilled water also stimulated the micoflora of the seeds in the case of untreated seeds (c) the presence of the fructifications of the micromycete *Cladosporium* spp. and in the case of the distilled water variant, the micromycete *Trichoderma* spp. was identified in one of the repetitive vessels. The presence of yeasts was also observed. The fructifications of the genera *Alternaria* and *Stemphylium* and

yeasts were identified on the MA culture medium when disinfected with 70% ethanol. In the Boema variety, micromycetes from the genera *Alternaria* and *Stemphylium* and *Penicillium* were identified in the seeds from all plates in the PDA variety. With the disinfection variant with distilled water, *Aspergillus* genus fructifications were also identified. In the case of disinfection with 70% ethanol, micoflora was poor, consisting of young *Alternaria* and *Stemphylium* fruits, the latter predominating, and yeasts. On the malt agar culture medium, the micoflora consisted of fungi of the same genera and on the 70% ethanol disinfection malt agar variant it was composed of *Alternaria*-poor micoflora and small yeast colonies. The characteristic fructifications of the *Rhizopus* micromycete were identified in all the analyzed variants.

Table 1 Micoflora associated with wheat seeds

Variants			The pathogen (9 days)							
			Rhizopus spp.	*Alternaria* spp. + *Stemphylium* spp.	*Cladosporium* spp.	*Epicoccum* spp.	*Trichoderma* spp.	*Penicillium* spp.	*Aspergillus* spp.	Yeasts
Glosa	PDA	N(c)	+++	+++	-	++	-	-		-
		AD	+++	+++	-	-	-	+		-
		E 70%	-	+++	-	-	-	-		
	MA	N(c)	+++	+++	++	-	-	-		-
		AD	++	++	-		+	-		+
		E 70%	-	+++	-	-	-	-	-	+
Boema	PDA	N(c)	+++	+++	-	-	-	+++	-	-
		AD	+++	+++	-	-	-	+++	++	-
		E 70%	-	+	-	-	-	-	-	+
	MA	N	+++	++	-	-	-	+++	+	-
		AD	+++	++	-	-	-	+++	+	-
		E 70%	+	+	-	-	-	-	-	++

+ - detected in one of the three repetitions; ++- detected in two of the 3 repetitions; +++- detected in the three repetitions
N(C)- control variant; AD- distilled water variant; E- 70% ethanol solution

The incidence of fungi identified in the monitored variants was also calculated (Table 2) and it was found that in the case of the Glosa variety, the highest incidence values, over 80%, were calculated for micromycetes from the genera *Alternaria* and *Stemphylium* in both culture medium in the control variants and disinfection with distilled water. In the disinfection option with 70% ethanol, the incident values were 33.33% on the PDA medium and 46.66% on the seeds placed on the MA culture medium.

The frequency of the micromycete *Trichoderma* spp. was 33.33% and 13.33% values were recorded for micromycetes *Cladosporium* spp., *Epiccocum* spp. The incidence of yeasts with a value of 13.33% was recorded on the malt agar culture medium. A low frequency, 6.66%, was determined in the case of the micromycete *Penicillium* spp. on the PDA culture medium.

Regarding the Boema variety, it was found that the highest incident values were calculated in the case of the micromycete *Penicillium* spp. with values over 73%, followed by the micromycetes *Alternaria* spp. and *Stemphylium* spp with frequency values between 6.66% and 33.3% on both culture media, with higher values for seeds not disinfected or subjected to a light disinfection. Determinations were also made on the germination of the analyzed seeds and it was found that this was not affected.

Table 2. Incidence of fungi detected on wheat seeds (9 days)

Variants	The pathogens	PDA			MA		
		N (c)	AD	E 70%	N(c)	AD	E 70%
Glosa	*Alternaria* spp. +*Stemphylium* spp.	80	86.66	33.33	86.66	53.33	46.66
	Cladosporium spp.	-	-	-	-	13.33	-
	Epicoccum spp.	13.33	-	-		-	-
	Trichoderma spp.	-	-	-	-	33.33	-
	Penicillium spp.	-	6.66	-	-	-	-
	Drojdii	-	-	-	-	13.33	13.33
Boema	*Alternaria* spp. +*Stemphylium* spp.	26.66	13.33	6.66	33.33	26.66	20
	Cladosporium spp.	-	-	-	-	-	-
	Epicoccum spp.	-	-	-	-	-	-
	Trichoderma spp.	-	-	-	-	-	-
	Penicillium spp	73.33	73.33	-	73.33	60	-
	Aspergillus spp.	-	13.33	-	13.33	6.66	-
	Yeast			6.66			26.66

CONCLUSIONS

The micoflora of the wheat seeds included species of the genera *Alternari*a and *Stemphylium* with the highest incidence values in the case of the Glosa variety, followed by micromycetes from the genera *Trichoderma*, *Cladosporium*, *Epicoccum*. The micoflora of the seeds of the Boema variety included *Penicillium* spp. with the highest values of the incident, followed by *Alternaria* spp. and *Stemphylium* spp. and *Aspergillus* spp. Disinfecting the seeds with 70% ethanol ensured a decrease in the presence of contaminated micoflora in the caryopsis of the monitored varieties.

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