

Mycobiota of rape seeds in Romania. I. Identification of mycobiota associated with rape seeds from different areas of Romania

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The spectrum of fungal diversity associated with rape seeds belonging to 33 cultivars (Alaska, Astrada, Astrid, Atlantic, Betty, Champlain, Chayenne, Dexter, Digger, Elvis, Eurowest, Finesse, Herkules, Hydromel, Hydromel-MA, Ladoga, Manitoba, Masa Rom, Milena, Mohican, Montego, Nectar, Ontario, Orkan, Perla (4 lots), Remy, Robust, Rodeo, Saphir, Tiger, Tiger CBC Lot ROM06-121-110, Triangle, Valesca, Vectra) and 2 hybrids (H-90-20-83, H-90-21-83) has been established by samples' macroscopical and microscopical analyzing, during 2006-2008, for the first time in Romania. The Ulster method on malt-agar and PDA culture media has been used, evaluating the percentage of fungal taxons present on/in rape seeds. The most important pathogenic fungi identified were: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers., *Rhizoctonia solani* Kühn, *Alternaria brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltshire and *Fusarium* spp. Also, a large quantities of some saprophytic fungi, as *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Rhizopus* have been recorded. These ones have been affected the health condition of rape seeds, suppressing their germination and other vital phenomena. Among potential antagonistic fungi the following genera have been isolated: *Chaetomium* (0-4%), *Trichoderma* (0-10%), *Aspergillus* (0-14%), *Penicillium* (0-100%). Some correlations and comparisons have been established between fungal diversity, their provenience, cultivars, culture media (Malt-Agar/MA, Potato-Dextrose-Agar/PDA) used. It has been evaluated the behaviour of rape cultivars and hybrids towards the main rape seed pathogens.

Key words: rape, mycobiota, seed-borne fungi, potential antagonistic fungi

INTRODUCTION

Rape is in Romania at present an extending crop in comparison with the years 2001-2005, when its area was in a significant decreasing. The largest area of rape crop has been recorded until now in 2010, the increasing of its surface starting since 2007 (Tab. 1). The highest values of the rape yields (569.6-943.0 thousands tones/ha) have been obtained during 2008-2010 and the lowest one (8.1-98.7 thousands tones/ha) during 2002-2004.

Regarding the average yield evaluated in kg rape seeds/ha, the highest seed production (1984 kg/ha) was recorded in 2004, followed by the values of 1357-1844 kg/ha between 2005-2010, with an exception (991 kg/ha) in 2007.

Table 1
Dynamics of rape crop areas and yields during 2001-2010 in Romania
(after the Statistical Yearbooks of Romania, 2007-2011)

Year	Crop area (thousands ha)	Yield (thousands tones/ha)	Average yield (kg/ha)
2001	82.4	101.0	1235
2002	74.6	35.9	481
2003	17.1	8.1	473
2004	49.7	98.7	1984
2005	87.8	147.6	1681
2006	110.1	175.1	1590
2007	364.9	361.5	991
2008	365.0	673.0	1844
2009	419.0	569.6	1357
2010	537.3	943.0	1755

■ the lowest values

In order to obtain a constant valuable rape yield it has to be applied in the crop technology the suitable agrotechnological and protection methods, providing a healthy crop developed from high quality seeds, free of seed-borne pathogens, with a high germinative faculty. Also, in the frame of the sustainable agriculture concept, it has to be substitute, as much as possible, the chemicals with the ecological, non-chemical products or with different other alternative non-polluting protection means (Baicu, Săvescu 1986; Hălmăjan et al. 2006).

These objectives have been followed during our researches, starting with an approach on endo- and epiphytic mycobiota of rape seeds belonging to various cultivars from different pedoclimatic zones of Romania.

In general, there are a few researches on seed pathology in Romania, like books of Hulea et al. (1973) or Raicu and Baciu (1978). Also, no papers referring to the mycobiota of rape seeds in Romania have been presented, but not published in extenso until now (Tabuc, Ştefan 2003; Şesan, Groza 2007; Şesan 2008). This paper is the first published contribution in the field of seed-pathology of rape in order to have an evaluation of rape seed-borne fungi/mycobiota as a reference point for obtaining a healthy and efficient, productive rape crop.

The objective of this study has been the investigation and evaluation the rape seed healthiness and detection of potential active antagonistic fungi against the main rape phytopathogens in order to be used in the future in their biocontrol.

MATERIAL AND METHODS

As a biological material a number of 39 rape seed samples have been analyzed: 8 samples from the Central Laboratory of Testing Quality of Seeds and Propagative Materials (CLTQSPM) Bucharest (South of Romania), 7 from the Agricultural Experimental Research-Development Station (AERDS) Secuieni, Neamț district (North-Est of Romania), 24 from the Agricultural Experimental Research-Development Station (AERDS) Caracal, Olt district (South of Romania). These samples belong to 33 rape cultivars (Alaska, Astrada, Astrid, Atlantic, Betty, Champlain, Chayenne, Dexter, Digger, Elvis, Eurowest, Finesse, Herkules, Hydromel, Hydromel-MA, Ladoga, Manitoba, Masa Rom, Milena, Mohican, Montego, Nectar, Ontario, Orkan, Perla (4 lots), Remy, Robust, Rodeo, Saphir, Tiger, Tiger CBC Lot ROM06-121-110, Triangle, Valesca, Vectra) and 2 hybrids (H-90-20-83, H-90-21-83), from the seed yields of 2006 and 2007 years.

Methods used for the identification of mycobiota associated with rape seeds were: macroscopical analyze, optical microscopy (microscope Olympus CX 41 from Japan, with a digital soft camera control panel SP-350), wet chamber and Ulster methods, on two culture media: Malt-Agar (MA) and Potato – Dextrose – Agar (PDA) (Neergaard 1979; Musket, Malone 1997; Machado et al. 2002).

The evaluation of the data has been done by the frequency (F) of seed- and soil-borne fungi associated with the rape seed samples (Tab. 2, Figs 1-3).

Fungal nomenclature has followed Index Fungorum (<http://www.indexfungorum.org>) and Dictionary of the Fungi (Kirk et al. 2008).

RESULTS AND DISCUSSION

The performed results on identification of mycobiota associated with rape seeds from different areas of Romania are presented in the Table 2, Figs. 1-3 and Plates 1-5.

The most important seed-borne phytopathogens for the rape seeds are: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers., *Rhizoctonia solani* Kühn, *Alternaria brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltshire, *Fusarium* spp.

Among these, the first significant phytopathogens (*S. sclerotiorum*, *B. cinerea*) haven't been identified in all analyzed seed samples from the yields of 2006-2007 years. Absence of these seed-borne fungi was correlated with the crop rotation, avoiding sensitive plants (sunflower, soybean, carrot, potato) to these pathogens in the same asolament and especially with the very dry climatic conditions during 2006-2007.

It has been identified in only one sample, cv. Eurowest, the fungus *Rhizoctonia solani* Kühn, with a low frequency of 1%.

Among soil-borne pathogens, there were identified *Fusarium* spp., with a high frequency until 51%, and average limits between 0.4 until 10.25%, present in 62.5% of seed samples. The highest level of *Fusarium* spp. has been detected on the Masa Rom cv. (51%), followed by Hydromel-MA cv. (26%), Elvis (22%) and then by the hybrid H-90-21-83 (12%). The lowest occurrence of *Fusarium* spp. (F = 1%) has been detected for the cultivars: Tiger, Dexter, Finesse and Herkules.

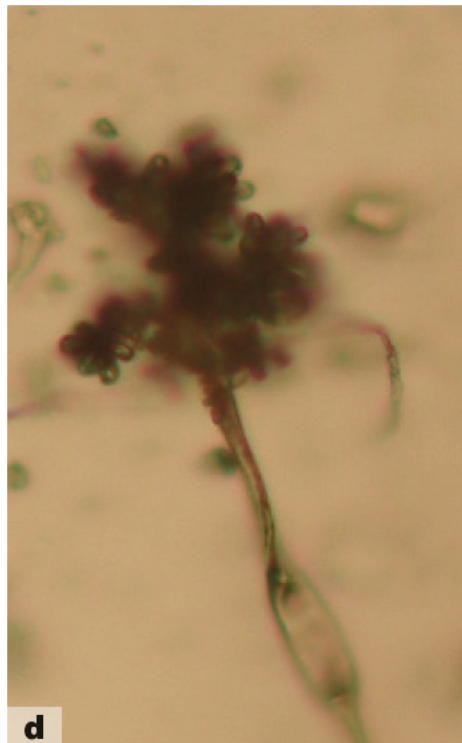
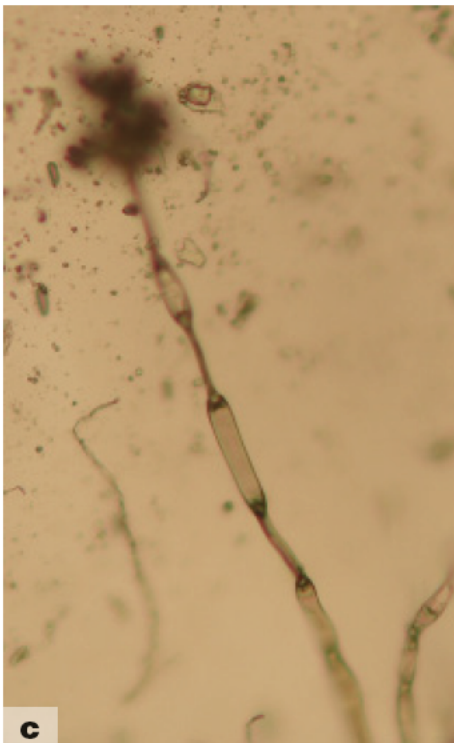
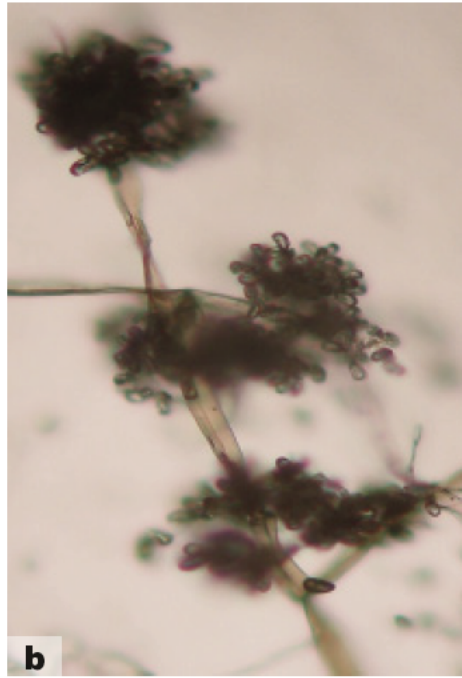
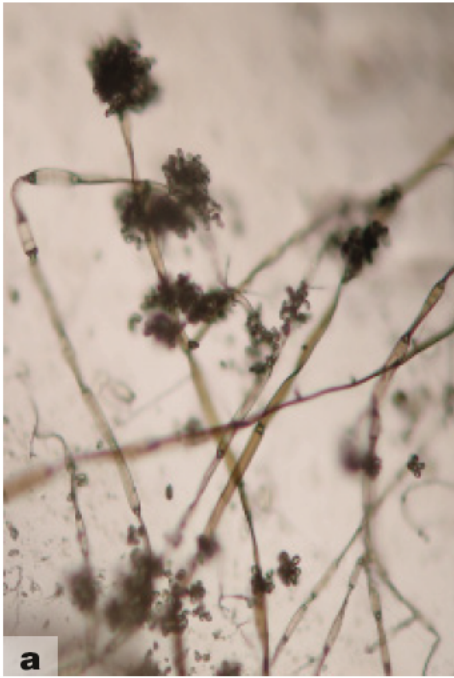


Plate 1. *Botrytis cinerea*: conidiophores and conidia; a-c – 20 x; b-d – 40 x.

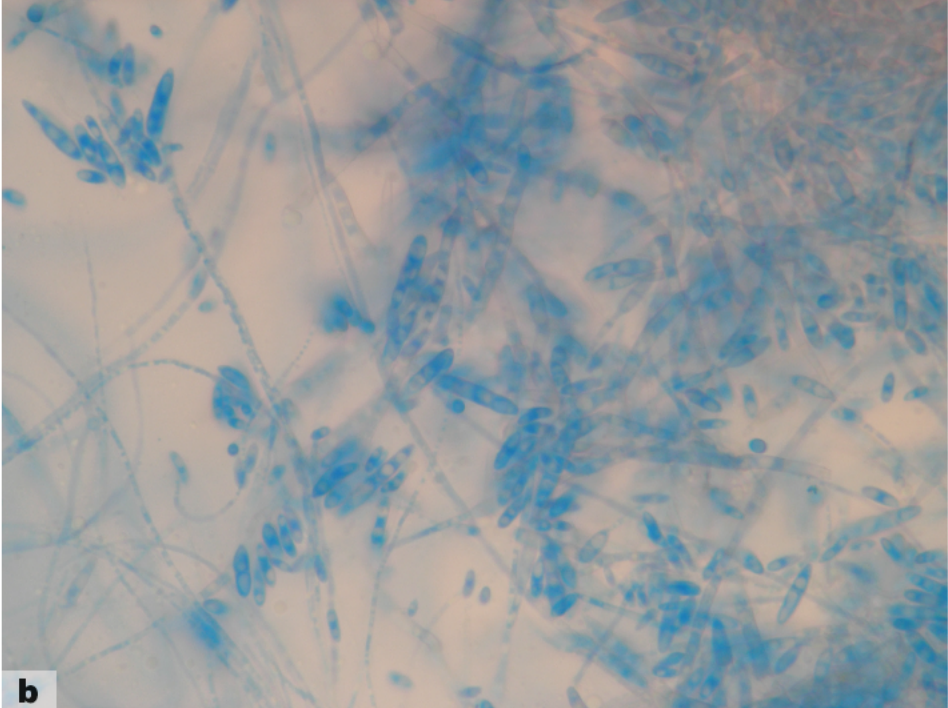
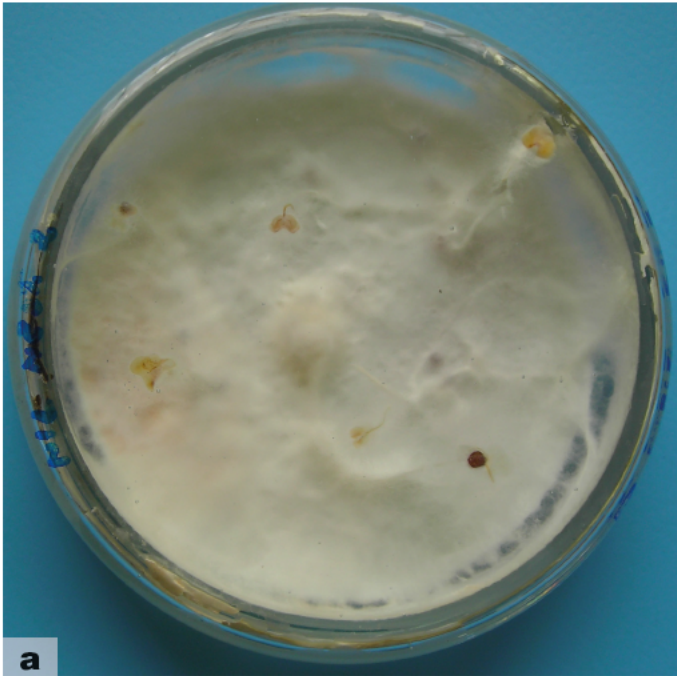


Plate 2. *Fusarium* spp.: a – Petri plate prepared by Ulster method for rape seed-borne mycobiota identification; b – conidiophores and conidia, coloured with bleau cotton (40 x).

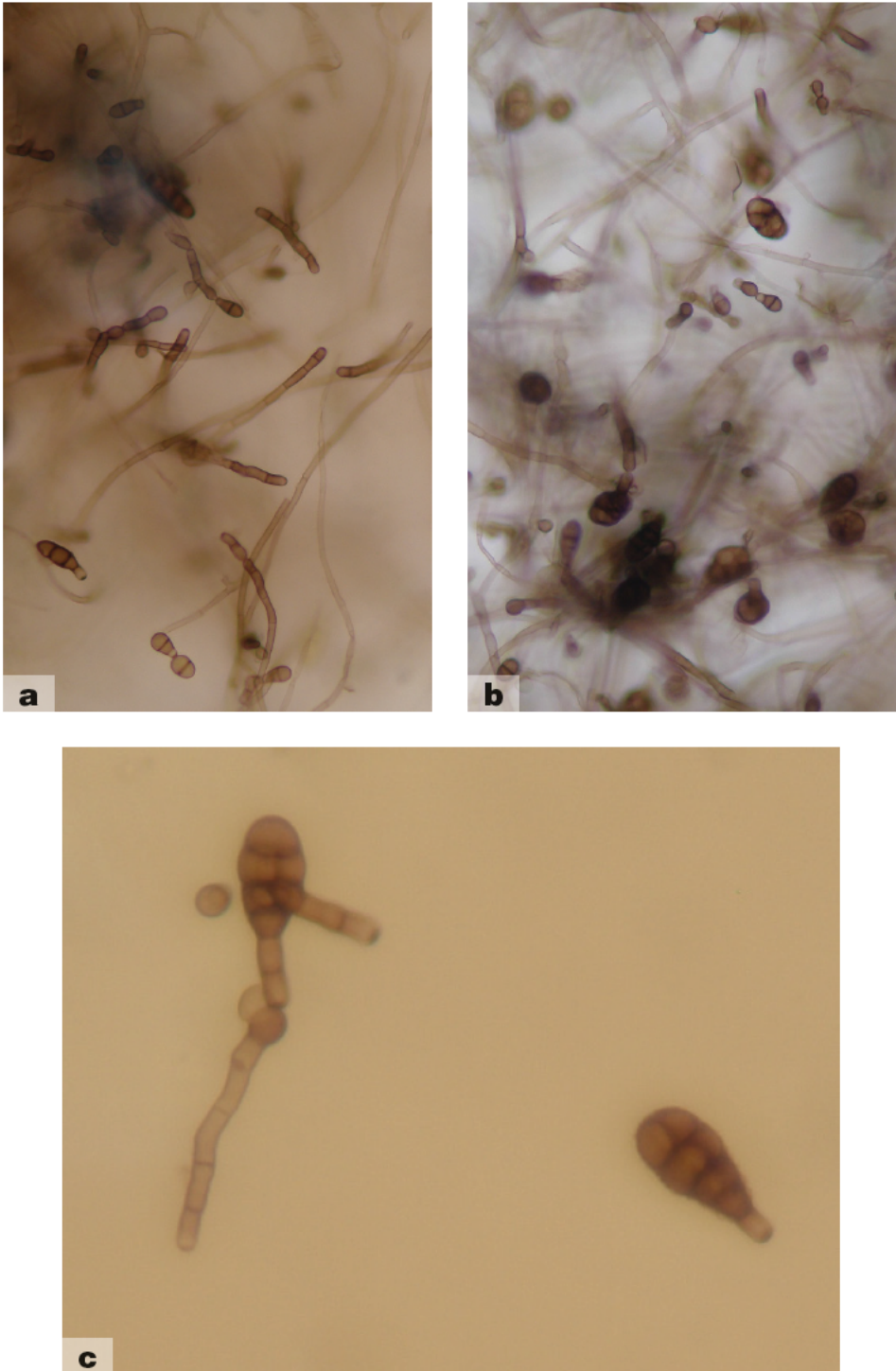


Plate 3. *Alternaria tenuis*: a-b – conidiophores and conidia (20 x); c – conidia (40x).

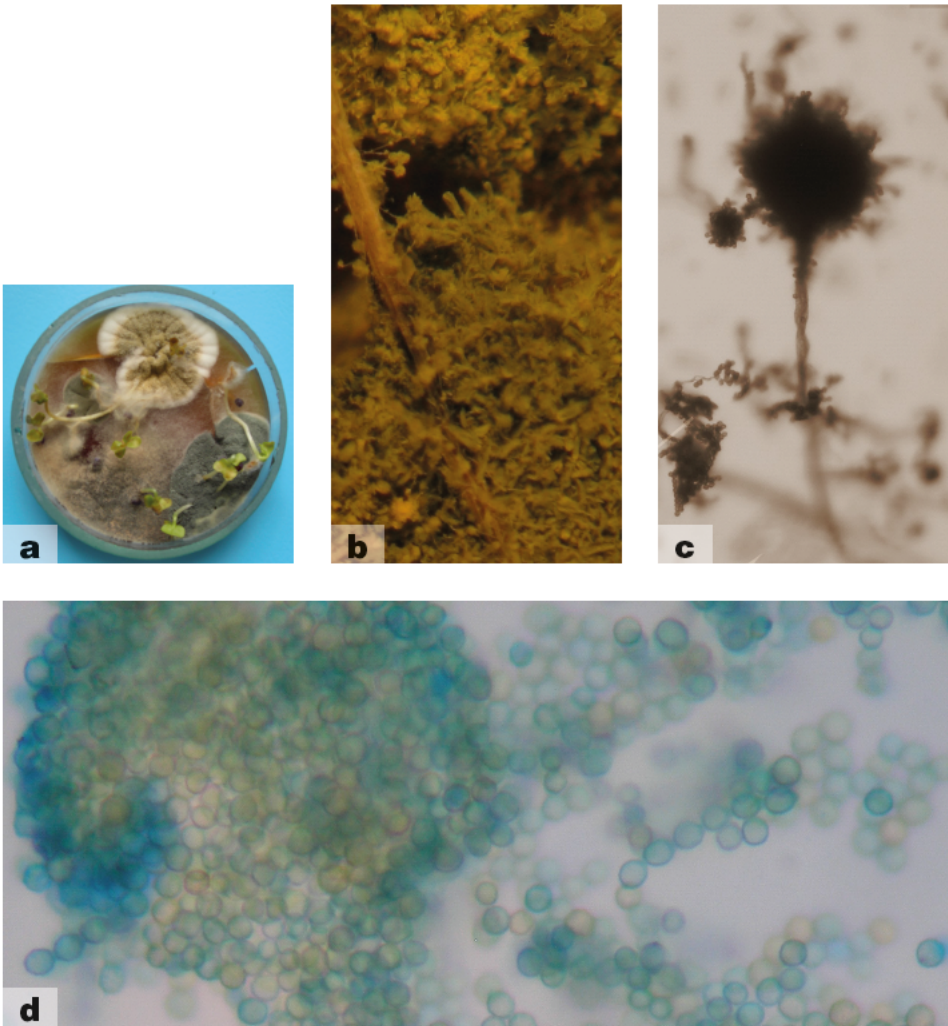


Plate 4. *Aspergillus ochraceus*: a – colony on PDA Petri plate; b – *A. ochraceus* – image under stereomicroscop with conidiophores and the conidia-forming tips; c – conidiophore and conidia (40x); d – conidia (60 x).

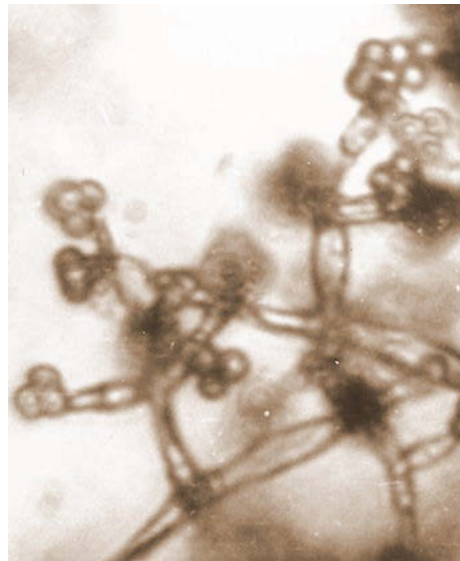
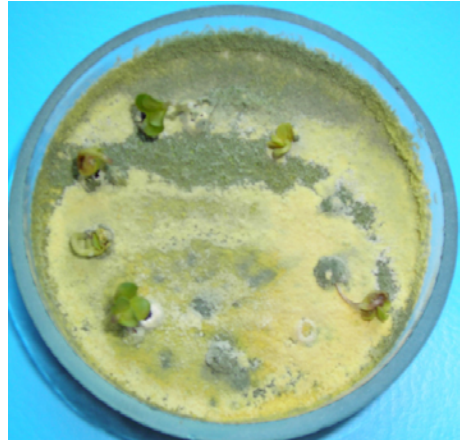


Plate 5. *Trichoderma* spp. Isolated from rape seeds: a-b – Petri plates prepared by Ulster method, where *Trichoderma* spp. has developed; c – conidiophores and conidia (10 x); d – idem (20 x).

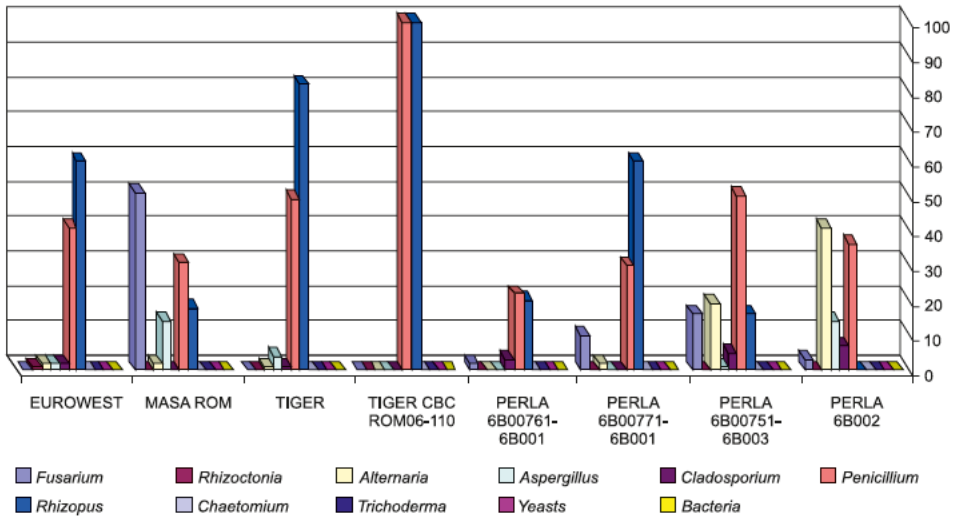


Fig. 1. Mycobiota frequency (%) from rape seeds on Malt-Agar (MA) medium (2007).

In 75% of the analyzed seed samples, there were identified species of *Alternaria*, with frequency until the highest value of 66%, and average limits between 6.3 until 15%. Among these species, the predominant one was *A. alternata* Fr.: Fr. Keissler (*A. tenuis* Nees). Among seed samples, the highest fungal presence of *Alternaria* spp. has been recorded for the Milena cv. (66%), followed by the hybrid H-90-21-83 and Perla cv. – Lot 6B002 (44-41%), and then by the cultivars Alaska (34%), Hydromel-MA (34%) and Robust (26%). The lowest percent of *Alternaria* has been recorded on the Tiger cv. (1%).

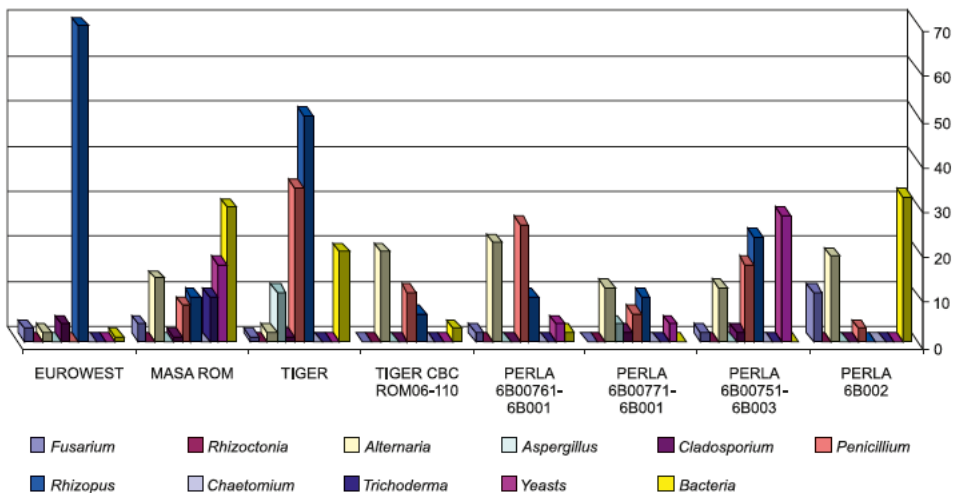


Fig. 2. Mycobiota frequency (%) from rape seeds on Potato Dextrose Agar (PDA) medium (2007).

Table 2
Main fungal pathogenic and saprotrophic species associated with rape seeds in România

No	Samples (I-III)/ Cultivars and hybrids (1-24)	Seed- and soil-borne fungi (%) detected on rape seeds														
		<i>Botrytis citra</i>	<i>Sclerotium sclerotiorum</i>	<i>Fusarium</i> spp.	<i>Rhizoctonia solani</i>	<i>Alternaria</i> spp.	<i>Aspergillus</i> spp.	<i>Cladosporium herbarum</i>	<i>Penicillium</i> spp.	<i>Rhizopus nigrans</i>	Other Mucoraceae	<i>Chaetomium</i> spp.	<i>Trichoderma</i> spp.	Yeasts	Bacteria	
I	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
	I.1	EUROWEST	-	-	1	2	2	2	41	60	-	-	-	-	-	-
	I.2	PERLA Lot 6B00751-6B003	-	16	19	1	5	50	15	1	-	-	-	-	-	-
	I.3	PERLA Lot 6B002	-	3	41	14	7	36	-	-	-	-	-	-	-	-
	I.4	MASAROM	-	51	2	14	-	31	18	-	-	-	-	-	-	-
	I.5	TIGER	-	-	1	4	1	49	80	2	1	-	-	-	-	-
	I.6	PERLA Lot 6B00761-6B001	-	2	2	-	3	22	20	-	-	-	-	-	-	-
	I.7	PERLA Lot 6B00771-6B001	-	10	2	-	-	30	60	-	-	-	-	-	-	-
I.8	TIGER CBC Lot ROM06-121-110	-	-	-	-	-	100	100	-	-	-	-	-	-	-	
	Average	-	-	10.2	0.1	8.4	4.4	2.2	45.0	44.1	0.4	0.1	-	-	-	
II. Potato Dextrose Agar medium (PDA)	II.1	EUROWEST	-	3	-	2	-	4	-	50	20	-	-	-	1	
	II.2	PERLA Lot 6B00751-6B003	-	2	12	-	2	17	20	3	-	-	-	28	-	
	II.3	PERLA Lot 6B002	-	11	-	19	-	3	-	-	-	-	-	-	32	
	II.4	MASAROM	-	4	-	14	-	1	8	10	-	-	10	17	30	
	II.5	TIGER	-	1	2	11	1	34	50	-	-	-	-	-	-	
	II.6	PERLA Lot 6B00761-6B001	-	2	22	-	26	10	-	-	-	-	-	4	2	
	II.7	PERLA Lot 6B00771-6B001	-	-	12	4	2	6	10	-	-	-	-	4	-	
	II.8	TIGER CBC Lot ROM06-121-110	-	-	20	-	-	11	6	-	-	-	-	-	3	
	Average	-	-	2.9	-	12.9	1.9	1.2	13.1	19.5	2.9	-	1.2	6.7	8.9	
III. on PDA medium	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	III.1	ASTRADA	-	-	-	-	11	1	4	6	-	-	-	-	7	-
	III.2	DEXTER	-	1	7	1	1	21	-	8	1	-	-	-	-	-
	III.3	FINESSE	-	-	1	8	4	25	15	-	-	1	-	-	-	-
	III.4	HERKULES	-	-	1	-	2	14	17	-	-	1	-	-	-	-
	III.5	ORKAN	-	-	-	6	3	3	20	-	-	-	9	-	-	-
	III.6	VALESCA	-	-	-	10	10	16	20	-	8	-	-	-	-	-
III.7	VECTRA	10	-	-	3	2	7	33	-	-	-	-	-	-	-	
	Average	1.4	0	0.4	0	6.3	3.3	10.0	19.0	0	1.1	0.2	2.4	1.1	0.1	

I		2		3	4	5	6	7	8	9	10	11	12	13	14	15	16
on PDA medium																	
IV.1	ALASKA	-	-	-	-	-	-	34	-	6	24	-	-	-	-	-	12
IV.2	ASTRID	-	-	2	-	-	-	-	-	4	-	98	-	-	-	-	12
IV.3	ATLANTIC	-	-	8	-	8	-	8	-	6	-	60	-	-	-	-	-
IV.4	BETTY	-	-	8	-	8	-	6	-	14	-	60	-	-	-	-	14
IV.5	CHAMPLEIN	-	-	2	-	2	-	-	-	-	6	60	-	-	-	12	-
IV.6	CHAYENNE	-	-	2	-	2	-	20	-	-	10	80	-	4	2	-	-
IV.7	DIGGER	-	-	2	-	2	-	22	-	-	10	60	-	-	-	-	-
IV.8	ELVIS	-	-	22	-	24	-	24	-	4	24	40	-	-	-	-	-
IV.9	H-90-20-83	-	-	2	-	2	-	10	-	6	6	80	-	-	-	-	-
IV.10	H-90-21-83	-	-	12	-	12	-	44	-	12	8	40	-	-	-	-	-
IV.11	HYDROMEL	-	-	2	-	2	-	-	-	8	2	40	2	-	-	-	-
IV.12	HYDROMEL-MA	-	-	26	-	26	-	32	4	4	-	40	-	-	-	2	-
IV.13	LADOGA	-	-	8	-	8	-	22	-	22	26	20	-	-	-	-	6
IV.14	MANITOBA	-	-	-	-	-	-	18	-	8	2	40	20	-	-	-	-
IV.15	MILENA	-	-	-	-	-	-	66	-	-	12	20	-	-	-	-	2
IV.16	MOHICAN	-	-	-	-	-	-	-	-	2	4	60	-	-	-	2	4
IV.17	MONTEGO	-	-	4	-	4	-	18	-	-	4	80	-	-	-	-	-
IV.18	NECTAR	-	-	4	-	4	-	-	-	1	2	100	-	-	-	-	-
IV.19	ONTARIO	-	-	4	-	4	-	-	-	14	-	100	-	-	-	-	-
IV.20	REMY	-	-	4	-	4	-	2	2	2	10	40	60	-	-	-	10
IV.21	ROBUST	-	-	12	-	12	-	26	-	-	2	18	-	-	-	-	-
IV.22	RODEO	-	-	6	-	6	-	-	-	-	-	100	-	-	-	-	8
IV.23	SAPHIR	-	-	-	-	-	-	-	-	2	8	100	-	-	-	-	2
IV.24	TRIANGLE	-	-	2	-	2	-	2	-	2	14	100	-	-	-	-	-
Average		0	0	6.0	0	6.0	0	15.0	0.25	5.0	6.0	60.0	3.4	0.2	0.08	0.7	3.0

Legend: **I – II** – seed samples from the CLTQSPMBucharest; **III** – idem from AERDS Secuient (Neamț district); **IV** – idem from AERDS Caracal (Olt district)

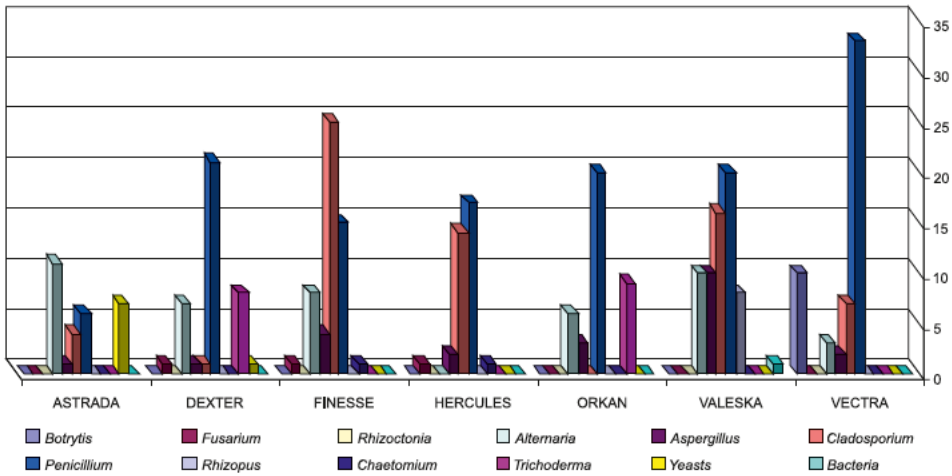


Fig. 3. Mycobiota frequency (%) on rape seeds from AERDS Secuieni - Neamt District (2008).

In almost all seed samples, epiphytic fungi have been identified. These ones, by their presence in such high level, increase the negative effects produced by phytopathogenic ones. These species were: *Aspergillus* spp. (F = 0-14%, mean limits between 0.25-4.4%), *Cladosporium herbarum* (Pers.) Link : S. F. Gray (F = 0-25%, mean limits between 1.2-10.0%), *Penicillium* spp. (F = 0-100%, mean limits 6-45%), *Rhizopus stolonifer* (Ehrenb.: Fr.) Lind. (F = 0-100%, mean limits 19-60%) and other fungi from Mucoraceae family (F = 0-60%, mean limits between 0.4-3.4%).

Among the potential antagonistic fungi, there were recorded from rape seeds: *Chaetomium* spp. (F = 0-4%, average limits between 0.1-0.2%), *Trichoderma* spp. (F = 0-10%, average limits 0.08-2.4%), and *Aspergillus* (F = 0-14%, average limits between 0.25-4.4%), *Penicillium* (F = 0-100%, average limits between 6-45%), already mentioned up, a.o.

Comparing the results performed on the two culture media, MA and PDA, it is evident that rape seed-borne fungi have grown better on MA and less on PDA (Tab. 2, I-II; Tab. 3).

Table 3
Comparison between occurrence of rape seed-borne mycobiota developed by Ulster method on MA and PDA culture media

Fungi	Limits of frequency (mean) %	
	MA	CGA
<i>Alternaria</i> spp.	1-41 (8.4)	2-20 (12.9)
<i>Aspergillus</i> spp.	1-14 (4.4)	4-11 (1.9)
<i>Cladosporium herbarum</i>	1-7 (2.2)	1-4 (1.2)
<i>Fusarium</i> spp.	2-51 (10.2)	1-11 (2.9)
<i>Penicillium</i> spp.	22-100 (45.0)	3-34 (13.1)
<i>Rhizopus stolonifer</i>	15-100 (44.1)	6-50 (19.5)
<i>Rhizoctonia solani</i>	1	-
<i>Trichoderma</i> spp.	-	0-10 (0.08-2.4)

In the performed investigations, *Rhizoctonia solani* have been identified on MA culture medium and it has not been recorded in our tests on the PDA culture medium. Also, in the Petri plates, on PDA medium, *Trichoderma* spp. have been identified. On the same culture medium (PDA) yeasts and bacteria associated with rape seeds presented a good development (Tab. 2, I-II) in comparison with MA medium.

These results explain why ISTA recommends Ulster method on the MA culture medium as a generalized standard method for seed pathology/ phytosanitary tests for the majority of cultivated plants (Malone, Muskett 1997; Machado et al. 2002).

Our results concerning the most important fungal phytopathogens of rape seeds – *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers., *Rhizoctonia solani*, *Alternaria* spp., especially *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltshire and *Fusarium* spp. – are similar with the results of other European scientists as Polish ones (Dakowska, Jędrzycka 2003; Jędrzycka et al. 2002, 2003; Sadowski et al. 2002a, 2002b). In addition, these results confirm the significance of these rape pathogens not only in Europe, but in the world, too (Agrios 2005; Saharagan et al. 2005; Cnola, Canada 2006).

CONCLUSIONS

1. The spectrum of fungal diversity associated with 39 rape seed samples belonging to 33 cultivars (Alaska, Astrada, Astrid, Atlantic, Betty, Champlain, Chayenne, Dexter, Digger, Elvis, Eurowest, Finesse, Herkules, Hydromel, Hydromel-MA, Ladoga, Manitoba, Masa Rom, Milena, Mohican, Montego, Nectar, Ontario, Orkan, Perla (4 lots), Remy, Robust, Rodeo, Saphir, Tiger, Tiger CBC Lot ROM06-121-110, Triangle, Valesca, Vectra) and 2 hybrids (H-90-20-83, H-90-21-83) has been established by samples' macroscopical and microscopical analyzes using Ulster method, during 2006-2008, in Romania.
2. The most important detected seed-borne phytopathogens for the rape seeds are: *Botrytis cinerea* Pers. (F = 10%, only on the Vectra cv.), *Rhizoctonia solani* Kühn (F = 1%, only on the Eurowest cv.), *Alternaria* spp., among them *Alternaria brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltshire (F = 0-66%) and *Fusarium* spp. (F = 0-51%).
3. Saprophytic seed-borne fungi recorded with the highest frequency on rape samples have been: *Cladosporium herbarum* (F = 0-25%), *Aspergillus* spp. (0-14%), *Penicillium* spp. (0-100%), *Rhizopus stolonifer* (0-100%), their presence affecting the seeds health status, mainly their germination faculty.
4. A strong contamination of rape seed samples from the South of Romania with *Rhizopus stolonifer* represents a risk factor in obtaining a healthy and highly productive rape crop.
5. Among the potential antagonistic fungi associated with rape seeds the following species have been detected: *Chaetomium* spp. (0-4%), *Trichoderma* spp. (0-10%), *Aspergillus* (0-14%), *Penicillium* (0-100%) a.o. This category of micromyceta are important in the selection of actives isolates for using them in biocontrol of phytopathogens.

6. Investigations performed on rape seed allow to evaluate the behaviour of 37 cultivars and 2 hybrids of rape to the main seed- and soil-borne fungi. It has been found 13 rape cultivars (Eurowest, Tiger CBC Lot ROM06-121-110, Astrada, Orkan, Valesca, Vectra, Alaska, Manitoba, Milena, Mohican, Saphir, Triangle) as resistant to *Fusarium* spp., 11 cultivars (Perla Lot 6B00761-6B001, Tiger CBC Lot ROM06-121-110, Herkules, Astrid, Champlein, Hydromel, Mohican, Nectar, Ontario, Rodeo, Saphir) resistant to *Alternaria* spp. and 9 cultivars (Astrada, Dexter, Finesse, Herkules, Orkan, Valesca, Vectra, Perla Lot 6B002, Alaska) resistant to *Rhizopus stolonifer*.

On the other hand, some cultivars proved sensitivity to the main seed- and soil-borne fungi: 3 sensitive cultivars (Masa Rom., Hydromel-MA, Elvis) to *Fusarium* spp., 4 cultivars (Milena, Perla Lot 6B00751-6B003, Alaska, Robust) and 1 hybrid (H-90-21-83) to *Alternaria* spp., and 6 cultivars (Ontario, Tiger CBC Lot ROM06-121-110, Nectar, Rodeo, Saphir, Triangle) to *R. stolonifer*. These data are very useful to the practice for selection of cultivars/hybrids in order to obtain a healthy and productive rape crop.

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