Research on micoflora present on rapeseed (*brassica napus*)
in the south region of Romania

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Abstract
The aim of this study was to estimate the distribution of Alternaria sp. present on seeds of *Brassica napus*. The samples of seeds were taken, just prior to harvest, directly from growing fields from Drajna (n=11), Borcea (n=9), Roseti (n=7), Tonea (n=3), Dragalina (n=1), Coslogeni (n=1), Dichiseni (n=1), Fundulea (n=1), Unirea (n=1).

Three replicates of five seeds from the same cultivars were incubated for 10 days, at 22°C on PDA. The semi-permanent slides were examined under a microscope (x40), field by field, until a total of 50 spores of Alternaria were identified.

*Alternaria brassicicola* was the most common (54.4%) fungus identified. In 45.7% of the plates the distribution of the six *Alternaria* species between colonies from the same plate is different. The distribution of each species of *Alternaria* in the growing fields around each of the four settlement for which multiple samples were available reveals a different distribution for *A. tenuis* in samples from Drajna (p<0,0001) and *A. radicinum* (p=0,006) or *A. dauci* (p=0,02) in samples from Tonea.

In the growing fields from Calarasi the most common Alternaria fungus is represented by *A. brassicicola* and the concomitant infection with *A. brassicicola*, *A. brassicae* and *A. radicinum* is a common phenomenon.

Keywords: *Alternaria* sp., rapeseed, fungus, distribution

1. Introduction

*Alternaria* sp. is a heterogen group of fungi, widespread distributed in nature (G. YAMH). Although host affinity is variable often they may be found in conjunction (M. NOWICKI, & al.). The pathogenic species can affect all parts of the plants and have important economical consequences (N.A. ANSARI, & al., A.E. PERELLÓ, & al.). Transmission of fungus through infected seeds may exacerbate these consequences S.K. SHRESTHA, & al.). In this context, development methods for identifying *Alternaria* sp. and characterization of coinfections are useful for disease prevention, field protection and epidemiological studies.

*Brassica napus* is an annual oil crop in the Brassica family used for flour, feed and oil production. The rapeseed acreage and production have shown significant variations in recent years in Romania. Calarasi County is one of the largest producers of rapeseed din Romania. In 2013, 46,859 ha were cultivated with rapeseed (~44% of the total surfaces cultivated with rape seed in our country) and the production was 140,096 tones. Although the conditions (e.g.
wind, rainfall) from Calarasi County may favor fungus development the data relating to the features of infections with Alternaria species and their economical impact are not available (S. CRISTEA, & al., C.M. CRISTEA, & al., M.S. CRISTEA, & al., M. PANĂ, & al.). Growing of varieties less susceptible to fungi Alternaria may represent an effective means in preventing infections, especially in more favorable years of the attack.

**Aim**

The aim of the research was to test the presence of *Alternaria* pathogens on *Brassica napus* seeds taken directly from growing fields from Calarasi County in 2014 and their distribution throughout the vegetative mass developed on culture medium in laboratory conditions.

### 2. Material and methods

The samples of seeds (n=35) were taken directly from growing fields from Calarasi county, in June (week 23-29), just prior to harvest. The growing fields are located surrounding to Drajna (n=11), Borcea (n=9), Roseti (n=7), Tonea (n=3), Dragalina (n=1), Coslogeni (n=1), Dichiseni (n=1), Fundulea (n=1), Unirea (n=1) villages. From each of the 35 growing fields we collected 0.5 kg of seeds. Monthly amount of rainfall during harvesting was between 76-125 mm.

![The map of Calarasi County.](image)

Five seeds from the same cultivars were placed on each Petri dishes (ø 10 cm) on potato-dextrose-agar culture medium, sterilized at 121°C/20 min (A. HULEA, O. CONSTANTINESCU). Three replicates were performed for each sample. The cultures were incubated for 10 days, at 22 °C. The Petri dishes were stored at 5°C until they were used to perform semi-permanent slides for microscope (x40) analysis. Microscopic analysis was extended until a total of 50 spores of *Alternaria brassicicola*, *A. brassicae*, *A. radicinum*, *A. tenuis*, *A. solani* and *A. dauci* have been identified (C. RAICU, & al.).

The data for the most common six *Alternaria* species identified were used for statistical analysis. The distribution of *Alternaria sp.* was compared with StatsDirect software (version 2.8.0) and a p values <0.05 have been considered statistical significant.
3. Results and Discussions

Thirty-five samples from Calarasi County were scored for this research. Vegetative growth was greatest after ten days. The colonies were blackish gray, fluffy appearance, with irregular edges and lighter brown and blackish reverse. A total of 27,828 spores of *Alternaria* sp. were investigated (Fig 2), of which 26,250 spores were identified with certainty (250 / plate) whereas 1,578 (5.67%) were excluded because the *Alternaria* species could not be established with certainty.

Microscopic analysis of semi-permanent slides performed from colonies developed in vitro revealed that for specific conditions to Calarasi in 2014 the most common six *Alternaria* species identified were *Alternaria brassicicola*, *A. brassicae*, *A. radicinum*, *A. tenuis*, *A. solani* or *A. dauci* (Table 1). In our samples the dominant *Alternaria* species was *Alternaria brassicicola* which represented 54.4% of all fungus identified.

![Fig 2 Mycelium of Alternaria sp. formed on PDA medium (light microscope, 40x).](image)

Table 1. Distribution of the most common six *Alternaria* species identified in samples collected in 2014 from 35 growing fields from Calarasi county.

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>A. brassicicola</th>
<th>A. brassicae</th>
<th>A. radicinum</th>
<th>A. tenuis</th>
<th>A. solani</th>
<th>A. dauci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>identified</td>
<td>14,274</td>
<td>4,647</td>
<td>3,465</td>
<td>1,845</td>
<td>1,764</td>
<td>255</td>
</tr>
<tr>
<td>(54.38)</td>
<td></td>
<td>(17.70)</td>
<td>(13.2)</td>
<td>(7.03)</td>
<td>(6.72)</td>
<td>(097)</td>
<td></td>
</tr>
<tr>
<td>Minimum/plate</td>
<td>(%)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Maximum/plate</td>
<td>(%)</td>
<td>41</td>
<td>21</td>
<td>26</td>
<td>18</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(82)</td>
<td>(42)</td>
<td>(52)</td>
<td>(36)</td>
<td>(24)</td>
<td>(12)</td>
<td></td>
</tr>
</tbody>
</table>

The distribution of the *Alternaria* species identified in samples from the 9 regions in Calarasi county showed significant differences (Chi-square = 1242.45, DF = 170, p <0.0001). In 48 of the 105 (45.7%) plates the distribution of fungus species between the five colonies formed per plate presented statistically significant differences (p<0.05). The seeds from these plates are from growing fields from Drajna (n=4), Roseti (4), Borcea (2), Tonea (2), Fundulea (1), Coslogeni (1), Unirea (1), Dragalina (n=1). The highest differences (p<0.001) are recorded for seeds harvested from two fields from Drajna and from the investigated growing field from Unirea.

Comparison of the distribution of each species of *Alternaria* in the 10 samples reveals that only *A. tenuis* was differently represented (p <0.0001). When the analysis was restricted to seeds harvested from growing fields around Unirea, Dichiseni, Fundulea, Coslogeni and Dragalina only *A. brassicae* has been found to present a different distribution (p<0.03).
The distributions of each *Alternaria* sp. in samples from neighboring growing fields (Drajna -11, Borcea -9, Roseti-7 and Tonea -3) were also compared. In this analysis, significant differences were noted for *A. tenuis* in samples from Drajna (p < 0.0001) and *A. radicinum* (p = 0.006) or *A. dauci* (p = 0.02) in samples from Tonea.

*Alternaria* sp. include saprophytic, endophytic and pathogenic species. Pathogenic species can have serious economic consequences on crops worldwide. The impact of these pathogens on crops in our country is not known.

In all investigated samples from Calarasi the most common *Alternaria* species detected were *A. brassicicola* (54.38%), *A. brassicae* (17.%) and *A. radicinum* (13.2%). The most common fungus, *A. brassicicola*, was present on each investigated seed and represented 20-82% from all Alternaria sp. scored. For 36 of the tested colonies (6.85%) the most common fungus was *A. brassicae* (24 seed) or *A. radicinum* (12 seeds). These are formed surrounding of seeds from Drajna (15 seeds from 4 fields), Roseti (15 seeds from 3 fields) and Borcea (6 seeds from 1 field), the fields which are not very close.

For 45.7% of plates the distribution of the six *Alternaria* sp. between the colonies from the same plate was different (p<0.05), because these seeds are from different growing fields (e.g. Drajna -4, Roseti -4, Borcea -2, Tonea -2, Fundulea -1, Coslogeni-1, Unirea-1, Dragalina-1). In addition in 45.7% of plates the distribution of the six *Alternaria* species between seeds from the same plate is different (p<0.05). These results suggested that the ratio between fungus varies in large limits in the neighboring and in distant growing fields.

**Table 2.** Distribution of the coinfections with two *Alternaria* species identified in all colonies developed surrounding of seeds from 35 growing fields from Calarasi county

<table>
<thead>
<tr>
<th></th>
<th><em>A. brassicae</em></th>
<th><em>A. radicinum</em></th>
<th><em>A. dauci</em></th>
<th><em>A. solani</em></th>
<th><em>A. tenuis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. brassicicola</em></td>
<td>99.43</td>
<td>95.43</td>
<td>30.29</td>
<td>90.29</td>
<td>74.86</td>
</tr>
<tr>
<td><em>A. brassicae</em></td>
<td>94.86</td>
<td>30.29</td>
<td>89.71</td>
<td>74.29</td>
<td></td>
</tr>
<tr>
<td><em>A. radicinum</em></td>
<td>29.14</td>
<td>29.14</td>
<td>85.71</td>
<td>70.86</td>
<td></td>
</tr>
<tr>
<td><em>A. dauci</em></td>
<td>29.71</td>
<td>29.71</td>
<td>21.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. solani</em></td>
<td>68.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We observed a great dispersion of several members of this group of fungi in the investigated area. *A. brassicicola* was observed on each seed, the presence of both *A. brassicicola* and *A. brassicae* was detected on 99.4% of colonies formed in vitro, whereas the presence of *A. brassicicola*, *A. brassicae* and *A. radicinum* was detected on 94.85% of colonies. It is worthy of mention that the most common four *Alternaria* species were detected in 29.14 of colonies and all the six *Alternaria* sp. were present on 20% of colonies formed in vitro. It becomes evident that in the agricultural areas from Calarasi the presence of *A. brassicicola*, *A. brassicae* and *A. radicinum* on infected seed is a common event whereas *A. tenuis*, *A. solani*, *A. dauci* have been more rarely detected. These results support the previous data regarding the variable affinity for the host of these funguses and the presence of mixt spores on seeds.

*A. brassicae* and *A. brassicicola* have been previously considered to have, independent or in conjunction, great economical impact on *Brassica sp.* (were concomitant presented). Although the frequencies of these two species on different parts of plants seems to be different (D. KUMAR D, & al.), in our study on 99.4% of tested seeds have been concomitant
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presented A brassicae and A. brassicicola (only one of these two species was found on only three cultivated seed of rapeseed). In this context we can speculate that different regional factors may promote the dispersion of *Alternaria* sp in the growing fields from Calarasi.

4. Conclusions

Our results demonstrated that in the growing fields from Calarasi in 2014 the most common *Alternaria* fungus was represented by *A. brassicicola* and the concomitant infection with *A. brassicicola*, *A. brassicae* and *A. radicinum* was identified in 94.85% of colony formed surrounding of seeds. Different local factors may also be responsible for spreading of these pathogens because in 45.7% of cases the seeds from the same field are different infected by these pathogens.

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References