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RESEARCH ON *ALTERNARIA* SPECIES PRESENT ON RAPESEED (*Brassica napus*) IN THE SOUTH REGION OF ROMANIA

Lavinia-Mariana BERCA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: laviniamariana.berca@gmail.com

Abstract

Alternaria species have a variable affinity for host although they are often found in conjunction. The aim of this study was to compare the distribution of six Alternaria species present in infected seeds samples taken directly from two districts in South Romania. Ten samples of seeds were taken directly from ten different growing fields in Teleorman and Calarasi, in June, just prior to harvest. For each of the ten samples has been performed three replicates, each of five seeds. Overall 150 seeds were placed on Petri dishes (ø 10 cm) on PDA. A semi-permanent slides was performed from each of the colonies formed around the seeds after 10 days of incubation, at 21ºC. These slides were analyzed, field by field, under a microscope (x40), until 50 conidia of Alternaria species were identified. For each replicate an average of 2350 conidia (150-250 conidia/ plate) were identified with certainty on 94% in vitro colonies formed by pathogens around the seeds. Alternaria brassicicola was the most frequent species identified on seeds from growing fields in Calarasi (53.91%) and Teleorman (57.12%). We found that A. brassicicola and A. brassicae were present on all infected seeds whereas A. brassicicola, A. brassicae and A. radicina were detected on 93.62% colonies formed around the seeds. The relative distribution of A. brassicae ($p < 0.0001$), A. brassicicola ($p < 0.0001$), A. radicina ($p = 0.001$) and A. solani ($p = 0.0003$) in samples from Calarasi and A. radicina ($p = 0.004$), A. solani ($p = 0.028$), A. tenuis ($p = 0.0006$) in samples from Teleorman were found be present statistical significant differences. Overall these results indicated that the distribution of Alternaria species can present significant variations even if samples from the same district are investigated.

Key words*: seeds, rapeseed pathogens, Alternaria, Romania.*

INTRODUCTION

Brassica napus ($2n = 38$) is cultivated in most European countries and performs well in a range of soil conditions, providing adequate moisture and fertility levels. The seeds are rich in proteins and oil (rapeseed contain an average of 40% oil on a dry matter basis) being used for rape oil. This plant is of interest for industry (humans or animals food industry, detergent lubrication oils, soap manufacture, production or erucic acid), medicine and research (Lim, 2013; Alvarez et al., 2001).

The seed yields vary from 900 to 3,000 kg/ha but decreases dramatically after infectious diseases (e.g. produced by fungi, bacteria, mollicutes, viruses,) and environmental damages (e.g. herbicide injury, nutrient deficiencies, postharvest disorders). Thus, diagnose and management of these diseases of *Brassica* species are of interest for farmers, pathologist, industry and seed providers (Rimmer, 2007; Afonin et al., 2008). *Alternaria* species is a diverse group of pathogenic and saprophytic fungi (Gherbawy, 2005) widespread distributed in soil and organic matter (Cristea et al., 2008; Cristea and Berca, 2013; Mardare et al., 2014; Pana et al., 2014; Cristea et al., 2015). Some *Alternaria* species were often found in conjunction in growing fields (Nowicki et al., 2012) and on *Brassica napus* seeds. The species responsible for Alternaria leaf spot disease can affect all stages of plants growth and have a wide range of negative effects on photosynthesis and production in wet seasons (Perelló et al., 2013; Shrestha et al. 2000). It is known that *A. brassicicola* and *A. brassicae* may be detected on surfaces and into the seed tegument and also in organic material (Rangel 1945; Humpherson-Jones, 1989, Humpherson-Jones and Phelps, 1989). The main ways of transport for pathogens are the diseased seed, while tools, animals, water and wind represent ways to disseminate the conidia.

Investigations of the relationship between different environmental factors and distribution of *Alternaria* species contribute to understanding disease epidemiology and measures necessary for prevention or protection of growing fields (Lou et al., 2013).
In Romania, rape area was 46,859 ha in the Calarasi County (2013) and 23,758 ha (2013), in the Teleroman County and represented 53.3% of the country' surfaces, with a production of 187,261 tonnes in 2013 (INSSE – Baze de date statistice). The wind and rainfall from agricultural areas from Teleorman and Calarasi can be permisive for *Alternaria* species development.
The aim of this study was to compare the distribution of six *Alternaria* species present in seed samples taken directly from two districts in South Romania.

MATERIALS AND METHODS

The seed samples were taken directly from ten growing fields with signs of alternariosis from Calarasi (Drajna-3, Roseti-1, Dichiseni-1) and Teleorman county (Draganesti Vlasca-2, Calomfiresti-2, Frasinet-1), in June 2014 (26-29 June), just prior to harvest (Figure 1).
Overall 150 seeds were used to perform three replicates for each of the ten samples. For each replicate five seeds from the same sample were placed on Petri dishes (ø 10 cm) on PDA culture medium (potato- dextrose- agar). The seeds have been not disinfected before this stage because we want to estimate the presence of *Alternaria* on seeds surface not into seeds tissues. The seeds were incubated for 10 days, at 21ºC (Hulea, 1969; Constantinescu, 1974). The in vitro colonies formed by pathogens were inspected and were white-gray and brown to black on the reverse side. The Petri dishes were stored at 5ºC until they were investigated.
A semi-permanent slide was performed for each colony formed around the seeds after 10 days of incubation.
The slides were examined under a microscope (x40), field by field, until 50 *Alternaria* spores were identified (Raicu and Baciu, 1978).
For each replicate an average of 2350 conidia (150-250 conidia/plate) was counted. The number of *Alternaria brassicae, Alternaria brassicicola, Alternaria radicina, Alternaria dauci, Alternaria solani* and *Alternaria tenuis* conidia identified on each slide was recorded.

Figure 1. The map of the South Region of Romania

The tables with distribution of *Alternaria* species was compared with χ^2 or Fisher test (rxc) using StatsDirect software (version 2.8.0). A p values < 0.05 was considered statisticaly significant.

RESULTS AND DISCUSSIONS

Overall, an average of 2350 conidia / replicate (150-250 conidia/plate) has been identified with certainty as *Alternaria* species on *in vitro* colonies formed by pathogens around the 94% of seeds (Figures 2 and 3). In addition the identity of 6.67% *Alternaria* species was not certain and they were excluded from analysis.


Figure 2. The Spores (conidia) of *Alternaria* species (light microscope, 40x)

The average of percentage distribution of the six *Alternaria* species in all colonies formed in *vitro* indicated that the predominant species are *A. brassicicola* (56%), *A. brassicae* (20%) and *A. radicina* (12%) while the other three species are rarer and have a frequency less than 10% (Figure 4).
The main results obtained on samples from each districts are presented in Table 1.

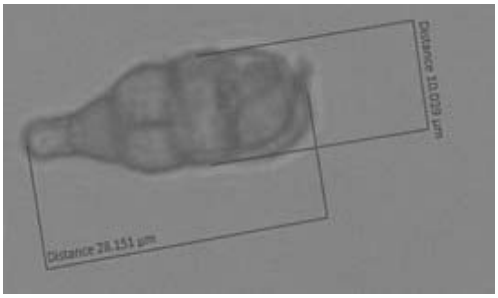

Figure 3. The Spores (conidia) of *Alternaria* species (light microscope, 40x)

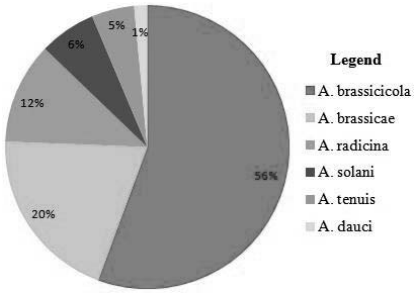

Figure 4. The percentage distribution of *Alternaria* species in 10 growing fields affected by alternaria leaf spot disease in Calarasi and Teleorman

When the relative distributions of the *Alternaria* species were compared in relation to provenience of seeds samples, the relative distribution of the six species was found to be similar and *Alternaria brassicicola* was the most frequent species identified in booth County (Calarasi: 53.91%, Teleorman: 57.12%).

The differences between relative distributions of these species are not significant. When the distribution of each species was analysed the highest differences between districts were identified for *A. brassicae* and *A. tenuis*.

Identification of what *Alternaria* species is more frequent on seeds it is important to establish the phytosanitary state of seed lot.

Next, the relative distributions of *Alternaria* species which are detected on the seeds taken from the same plate were tested.

The results presented significant differences in three samples from Teleorman and two samples from Calarasi (50% of samples). The highest differences ($p < 0.0001$) were recorded for the seeds harvested from Calomfiresti (Teleorman). The last test compared the distribution of each *Alternaria* species present on all seeds taken directly from growing fields from a single district. The results indicated that *A. brassicae* ($p < 0.0001$), *A. brassicicola* ($p < 0.0001$), *A. radicina* ($p = 0.001$) and *A. solani* ($p = 0.0003$) from Calarasi samples and A. *radicina* ($p = 0.004$), *A. solani* ($p = 0.028$), A. *tenuis* ($p = 0.0006$) from Teleorman samples were found to present a different distribution.

The results obtained from the last two tests indicated that the distribution of *Alternaria* species is influenced by different local factors.

Table 1. The percentage distribution of the six *Alternaria* species found on 150 seeds from ten growing fields in Calarasi and Teleorman

District	Parameter	*A. brassicicola*	*A. brassicae*	*A. radicina*	*A. solani*	*A. tenuis*	*A. dauci*
Calarasi	average %	53.91	22.09	11.09	7.45	3.64	1.82
	minimum %/plate	24	2	0	0	0	0
	maximum %/plate	76	46	30	20	10	14
Teleorman	average %	57.12	18.16	12	5.60	5.92	1.20
	minimum %/plate	24	4	0	0	0	0
	maximum %/plate	26	80	30		18	20

CONCLUSIONS

We found that fungus colonies surrounded 94% of the 150 seeds from growing fields affected by alternariosis. *A. brassicicola* (56%), *A. brassicae* (20%) and *A. radicina* (12%) are the most common species of *Alternaria* on these seeds.

Mixed infections with different *Alternaria* species seem to be common in investigated growing fields in Calarasi and Teleorman.

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