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Camelina Pathology-Seed Mycoflora Extension

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Abstract

Camelina sativa is a sustainable bioenergy crop, which represents a promising feedstock for the biofuel industry, and especially for the biojet fuel industry. The research was driven, as a complementary activity, during the ITAKA FP7, on cultivation of camelina varieties: Calena, GP202, GP204 and Camelia (Romanian variety) by identifying the camelina seeds mycopathogens within the Romanian climate and environment. The following pathogens were discovered: *Alternaria* spp, *Penicillium* spp., *Rhizopus* spp. and *Stemphilyum* spp., fungi that can influence the camelina seed yields and germination process. From the four tested varieties, the Romanian Camelia variety of camelina sp. was recognized as the most resistant for aggressive influence with less species` infection (only *Rhizopus* spp. with 32% incidence and respectively for *Alternaria* spp. with 68%) than the other varieties: Calena, GP202 and GP204. *Stemphylium* spp. pathogens are determined in association with *Alternaria* spp. for Calena, GP202 and GP204 varieties and the *Penicillium* spp. mycomycete had high incidence values on the GP202 and GP204 varieties. *Alternaria* spp. pathogens were present on each camelina variety studied and the pathogens association determined the influence on seeds germination and further on the infection of next crops. More studies are required to select specific suitable treatments.

Keywords: Biofuel, Camelina varieties, Mycoflora, Seed

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Introduction

Camelina`s crop represents an opportunity for sustainable agriculture as a double crop (Dobre et al. 2014) and a new alternative source for vegetable oil with industrial usage (Imbrea et al. 2011; Zubr 1997). Recently, camelina was studied for biofuels production as bio-kerosene or biodiesel (Shonnard et al. 2010; Tamba - Berehoiu et al. 2015; Frohlich et al. 2005). The plant and seed`s phytosanitary health represents an important factor for qualitative and increased yields (Raicu et al. 1978). During the cropping stage, the camelina plants can be infected by different pathogenic agents like *Alternaria* sp, & *Peronospora camelinae,* which determines a disease called mildew (Cristea et al.2014; Srivstava et al. 2012). Pathogenic agents belonging to the genera *Alternaria, Penicillium, Rhizopus, Stemphylium* are present frequently on the seeds surface (Cristea et al. 2008; Manole et al. 2015; Berca 2015) and they can also be detected on the camelina's seeds. The research regarding the identification of the spectrum of the pathogenic fungi on the camelina's seeds can be considered for the study of the qualitative indicators for this crop. The literature proves the existence of a significant high number of camelina genotypes, which can represent the basis of varieties selection with superior yield performance and oil content (Vollman et al 2007).

Materials and Methods

The research is targeted on the identification of the spectrum of pathogenic agents which are populating the camelina seeds. The biological material has been represented by untreated camelina seeds belonging to the varieties: Calena (Austrian variety), GP 202 & GP 204 (German variety) and Camelia (Romanian variety) (Toncea et al. 2013). The pathogenic agents` isolation has been done from seeds on Petri jars, on a PDA (potato-dextrose-agar) cultivation substrate and a successive replication of these. Pathogenic incubation has been done in a controlled temperature environment at 22 ^{0}C. The observations have been conducted at 3, 6, 9 days. The vegetative growth has been monitored and the pathogen sporulation has been observed. The fungi genera identification has been managed through microscopic observation, using a Zeiss Primo Star microscope, through a morphological analysis of the specific asexual fructifications (Barnett 1960).The statistical analysis of the experimental data was performed using ANOVA.

Results and Discussions

Pathogenic agents belonging to the genera *Alternaria, Penicillium, Stemphylium, Rhizopus* have been identified.

Table 1. *Fungi Detected on the Camelina Seeds Surface*

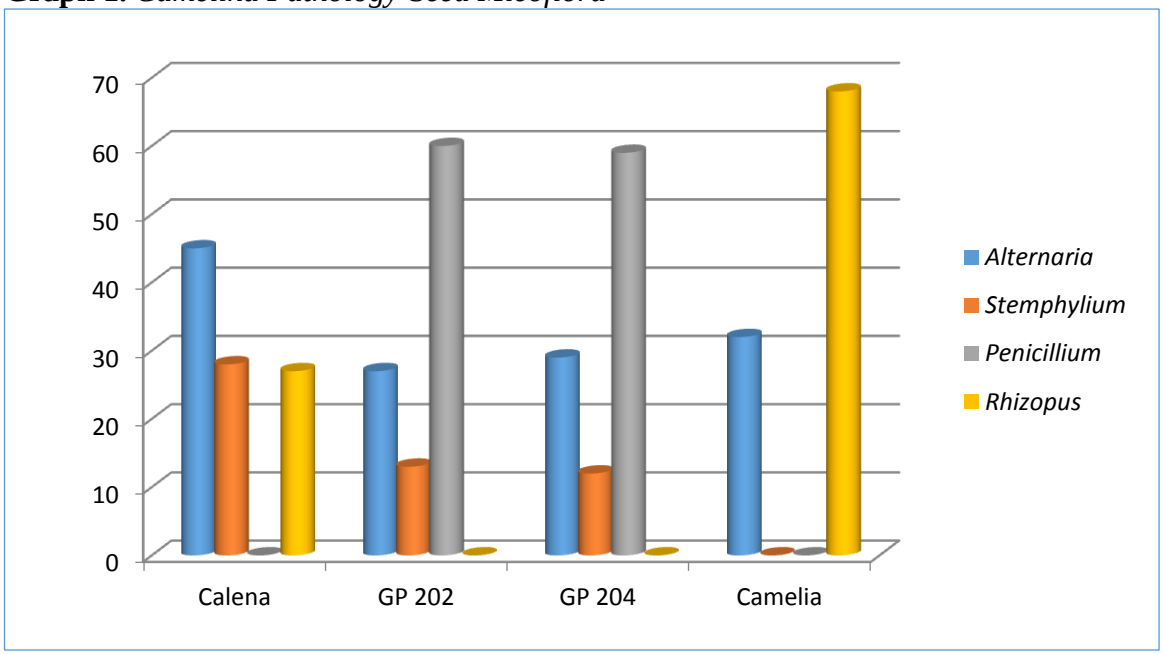
Variety	**Pathogenic agent**				
	Alternaria spp	*Stemphylium* spp.	*Penicillium* spp.	*Rhizopus* spp	*Fusarium* spp
Calena	+	+	-	+	-
GP 202	+	+	+	-	-
GP 204	+	+	+	-	+
Camelia	+	-	-	+	-

Data from Table 1 shows that on the surface of the camelina seeds belonging to the Calena variety fungi from the *Alternaria*, *Stemphylium* and *Rhizopus* genera have been detected and on the GP 202 seeds variety have fungi from the *Alternaria, Stemphylium* and *Penicillium* genra been identified.The GP 204 variety seeds have been populated with micromycetes from the *Alternaria* and *Penicillium* genera. The Camelia variety seeds have been showing micromycetes from *Alternaria* spp. and *Rhizopus* spp genera. In the case of the Camelia variety there; has been detected a narrower spectrum of pathogenic agents, comparative with the varieties Calena, GP 202 and GP 204. From the surface of the seeds belonging to the Camelia variety the pathogenic agents *Alternaria* spp. and *Rhizopus* spp. have been isolated. The fungi of genus *Alternaria* spp. have been present on the biological material from all the camelina varieties, which have been analyzed. The frequency of the identified micromycetes that has been determined for the analyzed varieties is highlighted in Table 2 and Graph 1.

Table 2. *Frequency of the Microflora Detected on the Camelina Seeds Surface*

Variety		**germinated seeds**		**Pathogenic agents**(after 9 days)									
				Alternaria spp.		*Stemphylium* spp.		*Penicillium* spp.		*Rhizopus* spp.		*Fusarium* spp.	
		%	*dif*	F (%)	*dif*	F (%)	*dif*	F (%)	*dif*	F (%)	*dif*	F (%)	*dif*
V1	Calena	100	Mt	45	Mt	28	Mt	-	Mt	27	Mt	-	Mt
V2	GP 202	97	-3	27	-18 ^{ooo}	13	-15 ^{ooo}	60	60***	-	-27 ^{ooo}	-	0
V3	GP 204	95	-5^{o}	29	-16 ^{ooo}	12	-16 ^{ooo}	57	57***	-	-27 ^{ooo}	2	2***
V4	Camelia	100	0	32	-13 ^{ooo}	-	-28 ^{ooo}	-	0	68	41***	-	0
	DL 5%		3.57		1.74		1.00		2.01		2.55		0.10
	DL 1%		5.62		2.84		1.67		3.36		4.21		0.17
	DL 0.1%		7.41		4.14		2.35		4.68		6.02		0.24

Graph 1. *Camelina Pathology-Seed Micoflora*



The identified micromycetes frequency from table 2 shows that the pathogenic agent *Penicillum* spp (Figure 1) displayed similar values of frequency for the GP202 and GP204 varieties (F= 60% and F= 57%). The presence of the fungi in the case of the Camelia variety hasn't been detected. The Camelia variety, displayed the highest value of frequency for the micromyceta species *Rhizopus* (F=68%) (Figure 2).

Analyzing the variety influence on germination of seeds of Camelina it is found that the percentage of germinated seeds ranged between 95 and 100%. Reported to the Calena variety which is the witness it is obseved that the two hybrids GP 202 and GPs 204 showed a reduction in the percentage of germinated seeds. This reduction is significant only for GP 204.

Figure 1. *Penicillium spp.- Sporulation (GP202 Variety)*

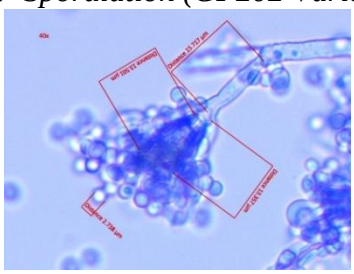
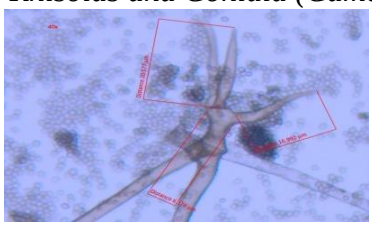
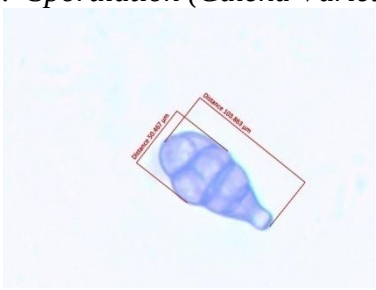


Figure 2. *Rhizopus spp.- Rhisoids and Conidia (Camelia Variety)*



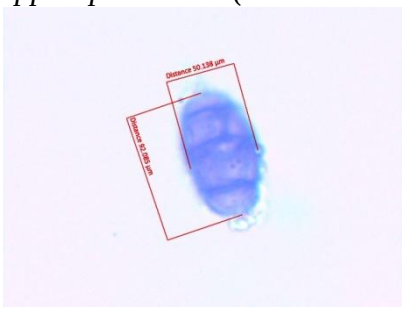
The frequency of the fungi from the genus *Alternaria* spp. has been showing high values in the case of the Calena variety, with a value of 45%, followed by the Camelia variety values of F=32%. The varieties of GP 202 and GP204 have displayed similar values of frequency in the case of the fungi belonging to the genus *Alternaria* spp. with a value of 27 % and respectively 29% (Figure 3).

Figure 3. *Alternaria spp.- Sporulation (Calena Variety)*



In what regards the frequency of the pathogenic fungi infection, from the genus S*temphylium* spp. (Figure 4), the data from the same table shows that its frequency values were 28% in the case of the Calena variety, and similar values of frequency for the GP202 and GP 204 varieties, of 13% and respectively 12%.

Figure 4. *Stemphylium spp.- Sporulation (GP 202 Variety)*



The seeds of the Camelia variety were free of *Stemphylium* spp., and the presence of the fungi *Fusarium* spp. being detected on the seeds surface of the GP204 variety, with a frequency of 2 % (Figure 1).

The seeds germination has not been affected in the case of the Calena and Camelia varieties. The lower germination values in the case of the GP202 and GP204 varieties can be due to the pathogenic agents' presence.

Conclusions

The camelina seeds mycoflora has been represented by fungi belonging to the genera *Alternaria*, *Penicillium*, *Rhizopus*, *Stemphylium*. The micromyceta of the genus *Alternaria* has been present on the seeds of all the studied varieties, the highest frequency recorded in the case of the Calena variety.The pathogenic agent *Stemphylium* spp. has been present together with the

Alternaria spp genus in the case of the Calena, GP202 and GP 204 varieties. Micromyceta *Penicillium* spp. has been present in the case of the GP202 and GP 204 varieties, with frequency values of 57 and 60%.The lower germination in the case of the GP202 and GP204 seeds varieties can be due to the pathogenic agents` presence on the seeds surface.

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