

RESEARCH ON MICROFLORA ASSOCIATED WITH ALFALFA SEEDS (*IN VITRO*)

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Abstract

The aim of the research was to identify fungi and the presence of other microorganisms on alfalfa seeds. The genetic material was represented by seeds of the Dobrogea genotype. We worked with three variants in three repetitions, untreated seeds (control variant), seeds passed through sterile water and disinfected seeds with 70% ethanol solution variant, under controlled conditions. Observations were made on microorganisms present on alfalfa seeds, under controlled conditions. The incidence of fungal microorganisms that developed on the culture medium, PDA (potato-dextrose-agar) was determined. Among the fungi, pathogens were detected: *Fusarium* spp., *Alternaria* spp., *Cladosporium herbarum*, *Aspergillus* spp., *Penicillium* spp. The highest incidence in the case of fungi was calculated in *Fusarium* spp. and *Aspergillus* spp. In the sterile water variant ($F = 22.2\%$). *Penicillium* spp and *Fusarium* spp pathogens were present in all variants studied.

Key words: alfalafa, pathogens, seeds, incidence.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is known as the oldest and most important perennial legume in the world. Lucerne is an important component of pastures in dry regions, rain-dominated areas and Mediterranean areas (Barbetii et al., 2006; Walsh et al., 2001; Piano and Francis, 1992). In some countries, alfalfa is the most important cultivated legume (Askar et al., 2012). The special importance of this plant is that it is a source of nitrogen fixation in the soil, provides a large amount of protein per hectare and an attractive honey plant for bees in obtaining honey (Stuteville and Erwin, 1990). Also, being a perennial plant, it can be exploited for 3-5 years and has a role as an ameliorating soil in the crop rotation. Alfalfa is the most important fodder crop in our country, representing 45.01% of the area cultivated with fodder plants (Schitea et al., 2020). Studies on the importance of crops and their market are important for agricultural research (Toth and Cristea, 2018, Popescu et al., 2018). Seed is an important means of transmitting plant diseases (Berca and Cristea, 2015; Dudoiu et al., 2016; Zaharia

et al., 2022). The transmission of diseases through the pathogen-carrying seed also implies an adequate management of their control. Alfalfa seed pathogenic fungi cause significant losses by reduced germination, seedling rot, seed rot (Couture et al., 2002; Krnjaja et al., 2003). *Fusarium* spp. causes root rot of seedlings and plant chlorosis of leaves and wilting of plants. Affected alfalfa seedlings show discoloration and damage to the roots that lead to seed rot and may contain mycotoxins (Goswami et al., 2008, Barbetti and Allen, 2005). The pathogens such as *Fusarium* spp., *Penicillium* spp. *Alternaria* spp. frequently populate seeds. Research on both foliar and seed diseases is relevant to alfalfa because some are transmitted to the crops with which it rotates (Barbetti et al., 2006).

MATERIALS AND METHODS

The research aimed to determine the spectrum of alfalfa seed microflora. The biological material was represented by seeds from the Dobrogea variety. The Dobrogea variety is a Romanian alfalfa variety, registered in 2019, in

Romania. We worked with three variants in three repetitions: variant one (V1) untreated seeds, variant two (V2) seeds disinfected with sterile water and then dried on filter paper and variant three (V3) seeds disinfected in 70% ethanol solution one minute and then passed through a sterile water bath and dried on sterile filter paper. The seeds were placed on the PDA-Roth medium in 90 mm Petri dishes, 6 seeds/Petri dish, in three repetitions. Seed were incubated at 22°C in a thermostat. The identification of the pathogens was made according to the morphological characteristics of the specific fructifications taken from the 6-day-old colonies. The Zeiss primo star microscope used to mount and the Zen software were used. In addition to fungi, the presence of other microorganisms, bacterial colonies and yeasts was noted. The incidence was calculated according to the formula $F = n \times 100/N$, where F = frequency (%), n = number of seeds with pathogen attack/variant, N = total number of seeds/variant.

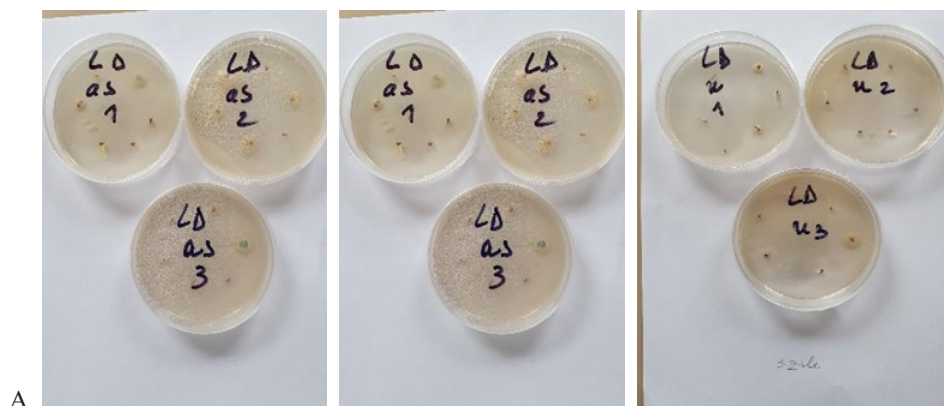
RESULTS AND DISCUSSIONS

The observations regarding the presence of pathogens on alfalfa seeds, Dobrogea variety presented in Table 1 show that pathogens belonging to the genus *Fusarium* were present in all analyzed variants. Also, microorganisms from the category of yeasts and bacterial colonies developed around the seeds in all the variants studied. Colonies of *Alternaria* spp. were identified in the sterile water and control variants and *Penicillium* spp. in the sterile water variant. Colonies belonging to the species *Cladosporium herbarum* were observed in the sterile water variant. The colonies of yeasts and bacterial microorganisms developed from the third day of observation. In the sterile water variant, a greater pathogenic diversity was found and the growth rate of the colonies was faster, which can be explained by the fact that a weakly activates the microbial flora (Figure 1: A and B). The spectrum of microorganisms that populate alfalfa seeds are found on the seeds of cultivated plants (Berca and Cristea, 2015; Cristea et al., 2015; Gheorghies et al., 2004). Also, the growth of microorganism colonies was faster compared to the other variants studied.

Table 1. Microflora associated with alfalfa seeds

Variety	Variants	The fungal pathogen/ 6 days					Other microorganisms	
		<i>Fusarium</i> spp.	<i>Alternaria</i> spp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Cladosporium herbarum</i>	Yeasts	Bacteria
Dobrogea	AS/E/C	+	+	+	+	+	+	+
	AS	+	+	+	+	+	+	+
	E	+	-	-	-	-	+	+
	C (untreated)	+	+	+	-	-	+	+

*AS=sterile water; E 70%= ethanol 70%; C= control



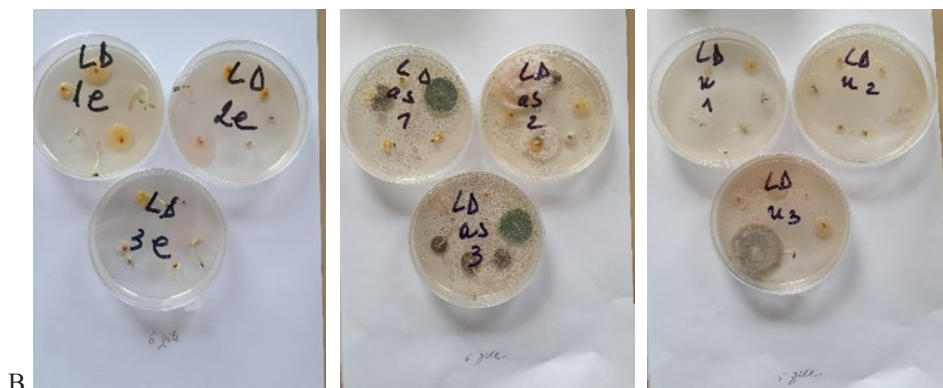


Figure 1. Microflora of alfalfa seeds, Dobrogea variety; variants: AS = sterile water; E 70% = ethanol 70%; C = control (A - 3days; B - 6 days)

The incidence of pathogens in total alfalfa seeds per variant was calculated and it was found that the most common microorganisms were yeasts and bacterial colonies, with 70% ethanol and control variants.

In the sterile water variant, the incidence of 22.2% was determined for the fungi *Fusarium* spp. and *Aspergillus* spp. (Figure 2).

In this variant, the presence of the fungi of the genera *Penicillium* and *Alternaria* was also determined, with a frequency of 11.1% (Figure 3, Figure 5).

In the 70% ethanol variant, *Fusarium* (Figure 4) pathogens were found to be present with a frequency of 11.1% and *Penicillium* spp with

5.5%. *Fusarium* species of fungi were generally found on alfalfa seeds (Askar et al., 2012, 2013). In this variant, the pathogenic spectrum is the poorest, which can be explained by the fungicidal action of the substance in the tested concentration. In the case of the (untreated) control variant, the frequency of *Fusarium* spp fungi was 22.2% and the fungi of the genera *Alternaria*, *Penicillium* and *Cladosporium herbarum* (Figure 6) were 5.5%. *Cladosporium* and *Alternaria* species are mentioned as being present on alfalfa seeds (EL-Garhy and EL-Wakil, 2014). Vasic et al., (2011) estimate that most fungi that affect alfalfa are transmitted through seeds.

Table 2. Incidence of alfalfa seed microflora

Variety	Variants	The fungal pathogen					Other microorganisms	
		<i>Fusarium</i> spp. F (%)	<i>Alternaria</i> spp. F (%)	<i>Aspergillus</i> spp. F (%)	<i>Penicillium</i> spp. F (%)	<i>Cladosporium herbarum</i> F (%)	Yeasts	Bacteria
Dobrogea	AS/E/C							
	AS	22.2	11.1	22.2	11.1	-	16.6	16.6
	E 70%	11.1	-	-	5.5	-	38.8	44.4
	Control (untreated)	22.2	5.5	-	5.5	5.5	16.6	44.4

*AS=sterile water; E 70%= ethanol 70%; C= control

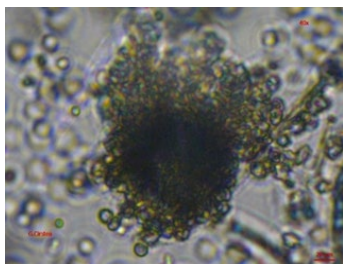


Figure 2. *Aspergillus* spp. (AS) (original)

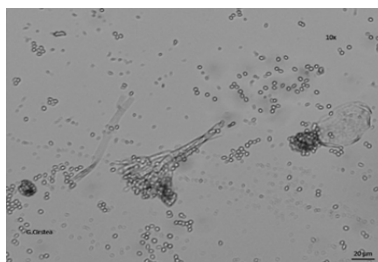


Figure 3. *Penicillium* spp. (AS) (original)

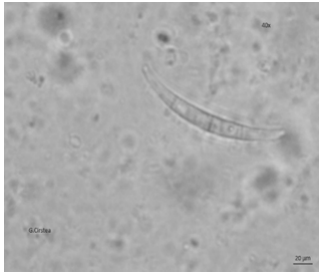


Figure 4. *Fusarium* spp. (etanol 70%) (original)



Figure 5. *Alternaria* spp. (AS) (original)

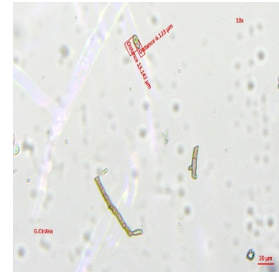


Figure 6. *Cladosporium herbarum* (control) (original)

CONCLUSIONS

Alfalfa seed microflora was composed of species of the genera *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Cladosporium herbarum*. Along with pathogens from the category of fungi, microorganisms belonging to yeasts and bacteria were also observed, which proved to be the most common. Among the fungi, the most common were those belonging to the genera *Fusarium* and *Aspergillus* (22.2% incidence).

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