

## EFFECTS OF PATHOGEN PRESENCE ON THE SEED QUALITY OF DIFFERENT ALFALFA CULTIVARS

### UTICAJ PRISUSTVA PATOGENA NA KVALITET SEMENA RAZLIČITIH SORTI LUCERKE

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#### ABSTRACT

Owing to its characteristics, alfalfa (*Medicago sativa* L.) is the most important forage plant in our country. The great importance of alfalfa is reflected in the production of high-quality fodder and alfalfa seeds, which are a valuable and competitive commodity on domestic and foreign markets. In this study, the presence of plant pathogenic genera of fungi on the seeds of five different alfalfa cultivars was investigated (Kruševačka 28, NS-Mediana, Zaječarska 83, Banjalučanka i Osječka-66) from three different lots (sites) of each cultivar. The detected phytopathogenic fungus on the alfalfa seeds caused a decrease in the overall germination, but the present research results indicate a satisfactory health status of all alfalfa cultivars and seed lots.

**Key words:** alfalfa, cultivars and seed lots, quality, pathogens.

#### REZIME

Plava lucerka (*Medicago sativa* L.) je zbog svih svojih osobina najvažnija krmna biljka u našoj zemlji. Veliki značaj lucerke se ogleda u proizvodnji kvalitetne krmne hrane, ali je i seme lucerke vredna i tražena roba na domaćem i inostranom tržištu. Areal gajenja lucerke je na svim kontinentima u više od 80 zemalja, od umereno hladnog do tropskog pojasa. Široka geografska rasprostranjenost lucerke uslovljena je njenom velikom adaptabilnošću na različite klimatske i zemljišne uslove. U ovom radu je ispitivano prisustvo fitopatogenih rodova gljiva na semenu pet različite sorte lucerke (Kruševačka 28, NS-Mediana, Zaječarska 83, Banjalučanka i Osječka-66) sa po tri različite partije (lokaliteta) od svake sorte. Kod ispitivanih sorti identifikovani su sledeći rodovi gljiva: *Alternaria* spp., *Fusarium* spp., *Penicillium* spp. i *Mucor* spp. Prisustvo identifikovanih rodova gljiva bilo je u rasponu od 0 % do 15,5%. Rod *Mucor* spp. najmanju prosečnu vrednost imao je kod sorte lucerke K-28 (0,08%), dok je najveću prosečnu vrednost imao kod sorte Zaječarska 83 (8,67%), a takođe kod iste sorte konstatovano je najveće prisustvo od (15,5%) na lokalitetu Veliki Izvor. Najveće prosečno prisustvo roda *Fusarium* spp. zabeleženo je kod sorte Zaječarska 83 (0,25%). Na proučavanim sortama lucerke ispitani su korelacioni odnosi između parametara kvaliteta semena i prisustva patogena na semenu različitih sorata i partija lucerke. Negativna korelaciona međuzavisnost zabeležena je između ukupne klijavosti i prisustva gljive iz roda *Fusarium* spp. ( $r = -0,415^*$ ). Detektovane fitopatogene gljive na semenu lucerke su uticale na smanjenje ukupne klijavosti, ali ipak rezultati ovih ispitivanja ukazuju na zadovoljavajuće zdravstveno stanje svih sorata i partija semena lucerke.

**Ključne reči:** lucerka, sorte i partije semena, kvalitet, patogeni.

#### INTRODUCTION

The production of alfalfa seeds in Serbia is significant for meeting domestic needs, but alfalfa seeds have also been exported in certain periods. The objective of alfalfa seed production is to achieve high seed yields and produce high-quality seeds (Đokić et al., 2011; Terzić et al., 2015). Alfalfa is invaded by a large number of pests and causal agents of plant diseases. Microorganisms which cause alfalfa root rot and wilting are the main agents responsible for a progressive decrease in the productivity of alfalfa. Many pathogens that cause diseases of aerial parts and roots of alfalfa are transmitted via infected seeds, thus seeds can be a very dangerous source of infection. Alfalfa seeds play an important role in the production of healthy plants because, if infected, they can carry spores of fungi which cause the rotting, reduced germination and total destruction of seedlings after germination (Abdul-Aziz et al., 2012).

Different fungi pathogens (*Phytophthora* spp., *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., *Colletotrichum* spp.,

*Verticillium* spp., *Sclerotinia* spp., etc.), responsible for certain types of alfalfa root and stem rot, may induce non-specific plant symptoms such as slower growth, chlorosis and wilt (Krnjaja et al., 2011). The symptoms present on alfalfa seeds, as well as on seeds of other forage plants, caused by pathogenic organisms may include seed rotting and seedling deterioration (Hanckok, 1983).

As a result of a large number of researches, nowadays the majority of alfalfa cultivars are characterized by a medium or high tolerance to pests and major causal agents of plant diseases, although the resistance has not been fully fledged and is still insufficient to protect alfalfa from the most resilient pathogens (Hill, 1987).

#### MATERIALS AND METHODS

This experimental research was carried out in the accredited laboratories for testing the quality of seeds and planting materials at the Institute for Plant Protection and Environment in Belgrade. Five (5) alfalfa cultivars of different geographic origin

(three different lots foreach cultivar)were used as research materials (Table1). The seed health was tested according to the Regulations on the Quality of Seeds of Agricultural Plants (Off. Gazette no. 47/87, 1987). For the purpose of testing, 4 x 100 seeds of each cultivar of alfalfawereused. The seeds were disinfected in 1% sodium hypochlorite (NaCl) for 10 minutes and placed in Petri dishes with a diameter of 90 mm. Filter paper was used as a substrate—soaked in distilled water until fully saturated. The samples were placed in a hotbed with a temperature of 20 °C and alternating light: 12 h without light and 12 h with ultraviolet light (UV) with a wavelength of 360 nm.

The testing was conducted after 7 days of seed incubation and the results of seed health surveys are expressed in the percentage of diseased seeds.

The experimental data obtainedwere processed using the statistical package STATISTICA 8.0 for Windows. The differences between the treatments were determined by the variance analysis (ANOVA).

Table 1. The origin of the alfalfa genotypes studied (*Medicago sativa* L.)

Cultivars	Origin	Lots/Sites		a.s.l. (m)
Kruševačka 28	Serbia	Banatsko Karadjordj.	45°34'19.70"N 20°34'31.28"E	73
		Ratari	44°21'05.92"N 20°51'03.35"E	114
		Niš	43°18'02.94"N 21°58'11.86"E	78
NS-Mediana 3MC V	Serbia	Vršac	45°04'12.22"N 21°33'38.96"E	182
		Bačko Gradište I	45°31'54.66"N 20°02'04.09"E	79
		Bačko Gradište II	45°32'53.23"N 20°00'34.35"E	76
Zaječarska 83	Serbia	Boljevac	43°49'49.08"N 21°57'11.16"E	284
		Veliki Izvor	43°57'40.14"N 22°23'36.63"E	320
		Minićevo	43°40'42.02"N 22°19'49.24"E	329
Banjalučanka	Republic of Srpska	Kozarska Dubica	45°11'04.30"N 16°48'23.97"E	103
		Banja Luka	44°40'24.21"N 17°34'44.69"E	274
		Maglajani	44°57'00.38"N 17°20'53.29"E	111
Osječka 66	Croatia	Istra	45°02'41.96"N 13°52'15.19"E	234
		Osijek I	45°31'36.12"N 18°27'02.20"E	88
		Osijek II	45°36'20.63"N 18°33'23.55"E	85

## RESULTS AND DISCUSSION

The assessment of the health status of tested alfalfa seeds was made on the basis of a seedling review. After seven days of incubation, the seeds indicated a visible mycelium of several species of fungi, but the presence of exudate in the form of small droplets wasnot observed. The determination of present fungi was based on morphological characteristics observed under a microscope.

Depending on the variety (average of the three lots-sites), the presence of the fungus *Alternaria* spp. on the seedlings was determined ranging from 0.08% in the alfalfa cultivar Kruševačka 28 up to 3.58% in the instance ofthe cultivar Zaječarska 83. The highest presence of this fungus was found on the seeds of the Zaječarska 83 cultivar, from the site of Veliki Izvor (6.75%). Conversely, this fungus was not generally detected in the majority of sites tested (Table 2). The fungus

*Fusarium* spp. was determined on the cultivars Zaječarska 83 (0.25%) and Kruševačka 28, but in the instance of the NS-Mediana and Banjalučanka seedlings it was not overalldetected. The pathogen was detected in the instance ofmost Osječka 66 seedlingsfrom the sites of Osijek I (0.50%). The seedlings of the Zaječarska 83 cultivar showed the highest susceptibility to the fungus *Mucor* spp. (8.67%), whereas the same species, from the site of Veliki Izvor, indicated the maximum percentage of detected fungi (15.5%). The lowest presence of the fungus was detected on the Kruševačka 28 cultivar seedlings 0.08%.

According to the rules on health examinations of crops and objects for seed production, seedlings and planting materials (Off. Gazette of RS, no. 119/2007), the following levels of alfalfa seed infection are approved (%): *Colletotrichum* spp. (1 %), *Fusarium* spp. (2 %), *Kabatiella caulivora* (2 %), *Sclerotinia* spp. (0 %), *Stemphylium* spp. (1 %), *Verticillium albo atrum* (1 %), alfalfa mosaic virus (AMV) (0 %), *Cuscuta* spp. (0 %).

Moreover, the analyses of seed health of six alfalfa cultivars (the seeds were disinfected using sodium hypochlorite (NaOCl)) confirmed the presence of fungi from the following genera:*Alternaria* spp.(0-5 %), *Cladosporium* spp.(0-1 %), *Fusarium* spp.(0-2 %) and *Stemphylium* spp.(0-1 %) (*Krnjaja et al., 2003*). On the basis of the colony morphology and the conidia analyses of three different varieties of alfalfa,the following genera of fungiwere identified: *Alternaria* spp.,*Fusarium* spp., *Penicillium* spp., *Mucor* spp. and steril mycelia. The presence of the identified fungi genera ranged from0% to 2% (*Štrbanović et al., 2013*).

Following the germination and seed health testingof five different alfalfa cultivars and lots,tge correlation coefficients (r) determined the relationship between the studied traits (Table 3).

The fungi detected on alfalfa seeds indicated a negative correlation interdependence with the overall

germination. A negative correlation was observed between the interdependence of the total germination and the presence of fungi of the genus *Penicillium* spp. ( $r = -0.415^*$ ). Moreover,the significant ( $P \leq 0.05$ ) and negative correlationswere obtained between*Alternaria* spp. and*Penicillium* spp. ( $r = -0.420^*$ ), while the negative interdependence was not statistically significant ( $P \geq 0.05$ ) between the germination andthe following fungi: *Alternaria* spp. ( $r = -0.130$ ), *Fusarium* spp. ( $r = -0.274$ ).

The most damaging pathogen of alfalfa seedswas the fungus *Fusarium oxysporum* f. sp. *Medicaginis*, causingalfalfa wilting(*Štrbac et al., 1996*).In alfalfa crops,*Fusarium* spp. causes the wilting of plant parts, but subsequently the pathogen induces the wilting of the whole plant(*Lević, 2008*). Considering the danger of spreading diseases through infected alfalfa seeds, it is important to use good quality and healthy seeds for sowing.

Table 2. The presence and variability of fungi identified on five different cultivars and alfalfa seed lots

Cultivars	Lots/Sites	Fungi (%)				
		<i>Alternaria</i> spp.	<i>Fusarium</i> spp.	<i>Penicillium</i> spp.	<i>Mucor</i> spp.	<i>Rhizopus</i> spp.
K-28	Ban. Karadjordjevo	0.25 a	0 a	0 a	0 a	0 a
	Ratari	0 b	0 a	0 a	0 a	0 a
	Niš	0 b	0 a	0 a	0.25 a	0 a
Average per lot/site		0.08	0	0	0.08	0
NS-Mediana	Vršac	0 b	0 a	0 b	0 b	0 a
	Bačko Gradište I	1.25 a	0 a	0 b	2.5 a	0 a
	Bačko Gradište II	0.5 b	0 a	0.25 a	0 b	0 a
Average per lot/site		0.58	0	0.08	0.83	0
ZA-83	Boljevac	2 b	0.25 a	0 a	8 b	0 a
	Veliki Izvor	6.75 a	0.25 a	0.25 a	15.50 a	0 a
	Minićevo	2 b	0.25 a	0 a	2.5 c	0 a
Average per lot/site		3.58	0.25	0.08	8.67	0
Banjalučanka	Kozarska Dubica	0 b	0 a	0 a	2.25 a	0 a
	Banja Luka	0.25 a	0 a	0 a	0 b	0 a
	Maglajani	0.5 b	0 a	0 a	0.5 b	0 a
Average per lot/site		0.25	0	0	0.92	0
OS-66	Istra	0.75 a	0 b	0 a	3.75 a	0 b
	Osijek I	0 b	0.5 a	0.25 a	0 b	0 b
	Osijek II	0 b	0 b	0 a	0 b	0 b
Average per lot/site		0.25	0.17	0.08	1.25	0

Table 3. The correlation coefficients (r) between the germination and fungi presence in the seeds of five different alfalfa cultivars

Trait	<i>Alternaria</i> spp.	<i>Fusarium</i> spp.	<i>Penicillium</i> spp.	<i>Mucor</i> spp.
Germination (%)	-0.130 <sup>NS</sup>	-0.274 <sup>NS</sup>	-0.415*	-0.225 <sup>NS</sup>
<i>Alternaria</i> spp.	-	-0.280 <sup>NS</sup>	-0.420*	-0.230 <sup>NS</sup>
<i>Fusarium</i> spp.		-	0.284 <sup>NS</sup>	0.248 <sup>NS</sup>
<i>Penicillium</i> spp.			-	0.026 <sup>NS</sup>
<i>Mucor</i> spp.				-

The level of significance: <sup>NS</sup>  $P \geq 0.05$ , \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ .

## CONCLUSION

The analyses of the health status of alfalfa seeds indicated no statistically significant differences between the cultivars observed in terms of the presence of the aforementioned fungi, but there were statistically significant differences between the seed lots. Regardless of the fungi presence, the percentage of germination in the examined alfalfacultivars meets the legal criteria stipulated in the Rules on the Health Condition of Seeds and Planting Materials. Considering severe damage fungi species can inflict to cultivated plants, the presence of pathogens on alfalfa seeds should be given more attention. In particular, it is

necessary to establish reliable methods of pathogen detection, including molecular methods, and monitor their development and expansion in alfalfa fields.

**ACKNOWLEDGEMENT:** This paper is a result of the research within the projects TR 31057 and TR 31018, supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Received: 23. 03. 2016.

Accepted: 24. 05. 2016.