

# Allelopathic tolerance of alfalfa (*Medicago sativa* L.) varieties to dodder (*Cuscuta epithymum* L.)

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## SUMMARY

Allelopathic effects of cold water extracts of *Cuscuta epithymum* L. on seed germination and initial development of *Medicago sativa* L. varieties were investigated under laboratory conditions at the Institute of Forage Crops, Pleven, during 2016-2017. It was found that the water extracts from dry biomass of *C. epithymum* had a considerably stronger inhibitory effect on the studied *M. sativa* varieties (IR 32.7-100.0 %), as compared to the extracts of fresh biomass (IR 0.2-40.5%). Depending on the kind of *C. epithymum* extract (fresh or dry parasitic weed biomass), IRs for seed germination of the tested *M. sativa* varieties could be conventionally classified into five groups: 1) seed germination stimulation, -1.4% – extracts from fresh biomass affecting the variety „Multifoliolate”; 2) seed germination inhibition of 0.1-10% – extracts prepared from fresh biomass affecting the varieties „Prista 3”, „Pleven 6”, „Prista 5” and „Obnova”; 3) seed germination inhibition of 11-20% – extracts from fresh biomass affecting the varieties „Roly” and „Victoria”; 4) seed germination inhibition of 30-45% – extracts from dry biomass affecting the variety „Multifoliolate”; 5) seed germination inhibition of 46-60% – extracts from dry biomass affecting the varieties „Prista 5”, „Prista 3”, „Victoria”, „Roly”, „Dara”, „Pleven 6” and „Obnova”.

The varieties „Victoria”, „Prista 5” and „Multifoliolate” of *M. sativa* possess some allelopathic tolerance because their germination indexes (GI) range from 80.5 to 88.7 % for the extracts prepared from fresh weed biomass of *C. epithymum*, and from 47.1 to 48.6% for the extracts from dry weed biomass, compared to control treatment. These varieties can be used as components in future breeding programmes.

**Keywords:** *Medicago sativa* L.; *Cuscuta epithymum* L.; Allelopathy; Seed germination; Inhibition

## INTRODUCTION

In the last few decades, research work has been focused on studying allelopathic interrelations between cultivated plants and weed species with the purpose of finding varieties with high allelopathic potential (Masum et al., 2016;

Trezzi et al., 2016). The discovery of varieties with high allelopathic potentials provides a possibility for decreasing inputs in agricultural crop growing and for producing organic foods (Bashir et al., 2011; Bashir et al., 2012).

According to several authors (Okuno & Ebana, 2003; Bertholdsson & Tuveesson, 2005; Jabran & Farooq, 2013)

allelopathic tolerance can be considered as a means for breeding programmes for biological control of weeds.

Weed infestation of alfalfa crops in Bulgaria is of a mixed type with predominating early- and late-spring weeds, perennial mono- and dicotyledonous weeds and some parasitic weed species, which deteriorate the quality of the obtained produce and decrease fodder and seed production (Dimitrova, 2005; Dimitrova & Serafimov, 2007a; 2007b; Dimitrova, 2008).

Literature data have revealed differences among the varieties of different crops (*Avena sativa* L., *Glycine max* (L.) Merr., *Pisum sativum* L., *Sorghum sudanense* Piper Stapf, *Triticum* spp., etc.) regarding their allelopathic tolerance to different weed species (Rice, 1995; Miralles et al., 2012; Fragasso et al., 2013; Cheng & Cheng, 2015). In Bulgaria, no detailed results have been published about allelopathic tolerance of Bulgarian alfalfa varieties to different weed species. The objective of this study was to determine allelopathic effects of cold aqueous extracts of fresh and dry biomass of dodder (*Cuscuta epithymum* L.) on seed germination, and growth and development of seedlings of different alfalfa (*Medicago sativa* L.) varieties, and to identify the varieties with allelopathic tolerance of that parasitic weed.

## MATERIAL AND METHODS

The study was conducted under laboratory conditions at the Institute of Forage Crops in Pleven, Bulgaria, during 2015-2016.

### Collection and preparation of plant material

Seeds of several alfalfa (*M. sativa*) varieties („Pleven 6“, „Dara“, „Obnova“, „Victoria“, „Prista 3“, „Prista 5“, „Roly“ and „Multifoliolate“) were sampled from operational collections of the Institute of Forage Crops, Pleven, and the Institute of Agriculture and Seed Science „Obraztsov Chiflik“, Ruse.

The biomass of plant samples of *C. epithymum* was collected in a weed-infested natural environment in the environs of the Institute of Forage Crops, Pleven, at the growth stage BBCH – 65-67 (Hess et al., 1997).

### Plant extracts

Aboveground biomass of *C. epithymum* was chopped into 0.5-3.0 cm long segments. Two kinds of weed extracts were prepared: A – from fresh weed biomass of *C. epithymum*, crushed with quartz sand, and B – from dry weed biomass after drying the samples to constant

dry weight at  $55 \pm 2$  °C and grinding in Retsch SM-1 grinder to a sieve size of 1.0 mm.

A hundred grams of dry and the same amount of fresh biomass of *C. epithymum* were soaked in 1 l<sup>1</sup> distilled water. The samples were prepared in such a way that fresh or dry biomass of *C. epithymum* were cold extracted at a temperature of  $24 \pm 2$  °C for 24 h in a shuttle apparatus at 150/60 c<sup>-1</sup>. The obtained extracts were decanted and filtered through filter paper. All available aqueous extracts were brought to weed biomass content of 25, 50 and 100 g biomass per litre of distilled water (presented hereinafter as g/l<sup>1</sup>) (Faravani et al., 2008). Thymol (C<sub>10</sub>H<sub>14</sub>O) was added to each extract as a preserving agent (Marinov-Serafimov et al., 2007).

### Bioassay

One hundred seeds of each alfalfa (*M. sativa*) variety („Pleven 6“, „Dara“, „Obnova“, „Victoria“, „Prista 3“, „Prista 5“, „Roly“ and „Multifoliolate“) were put in Petri dishes (90 mm diameter) between filter papers. All prepared extracts, according to their parasitic weed biomass contents, were pipetted at a ratio of 1:20 as against the seed mass (Marinov-Serafimov et al., 2007). Distilled water was used for control seeds. Each variant was laid out in ten replications. The samples were then placed in a thermostat-operated device at a temperature of  $22 \pm 2$  °C for seven days.

### Effect assessment

For assessing the experimental results the following biometric parameters were examined: seedling length (mm), and fresh weight of seedlings (g). Seedling length was measured using graph paper, while weight was measured on an analytical balance.

### Statistical evaluation and calculation formulas

Germination of seeds (GS<sub>%</sub>) was determined by Equation (1), according to ISTA (1985).

$$GS_{\%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds plated}} \cdot 100 \quad (1)$$

Inhibition rate (IR, %) was determined by Equation (2) (Ahn & Chung, 2000).

$$IR = \left[ \frac{C - T}{C} \right] \cdot 100 \quad (2)$$

where *C* – characteristic in the control treatment; *T* – characteristics in each treatment.

The index of plant development, i.e. the germination index ( $GI$ ), was determined by Equation (3) (Gariglio et al., 2002).

$$GI = \left[ \left( \frac{G}{G_0} \right) \cdot \left( \frac{L}{L_0} \right) \right] \cdot 100 \quad (3)$$

where  $G$  - germinated seeds in each treatment, %;  $G_0$  - germinated seeds in the control treatment, %;  $L$  - average length (cm) of seedlings in treatment transformed into percentage as against the control treatment;  $L_0$  - average length (cm) of the seedlings in the control treatment taken as 100%.

The percentage of seed germination in each treatment was previously transformed by Equation (4) (Hinkelman & Kempthorne, 1994)

$$Y = \arcsin \sqrt{\left( \frac{X\%}{100} \right)} \quad (4)$$

where  $x\%$  - germinated seeds for each treatment (%).

The collected data were analyzed in Statgraphics Plus for Windows Ver. 2.1 and STATISTICA Ver. 10.

## RESULTS AND DISCUSSION

The species and concentrations of the applied extracts of *C. epithymum* had a substantial influence on laboratory seed germination of the studied alfalfa varieties (Table 1). With increasing concentrations there was a general tendency of germination decrease in all varieties, i.e. 0.5-59.6% on average, as against the control treatment.

The extract of *C. epithymum* dry biomass showed a considerably stronger inhibitory effect on the studied alfalfa varieties (IRs 32.7-100.0%) and the differences are statistically significant at  $P=0.05$ , as compared to the extract of fresh biomass (IRs 0.2-40.5%) (Table 1). That was probably due to the considerably higher concentrations of that type of extract, compared to concentrations found in agrophytocenoses with falling off and decomposition of weed biomass. An exception was only the extract of 25 g l<sup>-1</sup> parasitic fresh weed biomass of *C. epithymum* in the varieties „Pleven 6“ and „Prista 3“, which showed a weak stimulating effect (from 1.4 to 8.2%) but the differences were not statistically significant (Table 1).

**Table 1.** Effects of different concentrations of aqueous extracts of *C. epithymum* on seed germination of *M. sativa* varieties, %

Variety	Contents of weed biomass in water extracts, g l <sup>-1</sup>									
	0		25		50		100		Average	
	GS <sub>%</sub>	IR	GS <sub>%</sub>	IR	GS <sub>%</sub>	IR	GS <sub>%</sub>	IR	GS <sub>%</sub>	IR
Fresh biomass										
Pleven 6	56.8a	0.0	65.5b	-15.3	55.3a	2.6	48.5a	14.6	56.5a	0.5
Dara	71.6c	0.0	63.4b	11.4	61.8b	13.7	56.8a	20.6	63.4b	11.4
Obnova	67.5b	0.0	63.4b	6.0	67.5b	0.0	53.8a	20.3	63.1b	6.6
Victoria	49.4a	0.0	49.3a	0.2	36.7a	25.7	29.4a	40.5	41.2a	16.6
Prista 3	53.8ab	0.0	56.8bc	-5.6	50.8a	5.6	11.55c	1.12	43.2a	0.3
Prista 5	53.8b	0.0	50.8ab	5.6	52.3ab	2.8	49.3a	8.3	51.6ab	4.2
Multifoliolate	60.1a	0.0	60.1a	0.0	61.8a	-2.8	61.8a	-2.8	61.0a	-1.4
Roly	63.4d	0.0	58.5c	7.9	53.8b	15.2	45.0a	29.1	55.2b	13.1
Average	59.6d	0.0	58.5c	1.3	55.0b	7.9	44.5a	16.5		
Dry biomass										
Pleven 6	56.8c	0.0	29.9b	47.4	3.7a	88.2	0.00a	100.0	22.6b	58.9
Dara	71.6c	0.0	42.1b	41.2	8.3a	88.4	0.00a	100.0	30.5b	57.4
Obnova	67.5c	0.0	33.2b	50.8	8.3a	87.7	0.00a	100.0	27.3b	59.6
Victoria	49.4c	0.0	28.3b	42.7	16.6ab	66.4	0.00a	100.0	23.6b	52.3
Prista 3	53.8d	0.0	36.2c	32.7	13.3b	75.3	0.00a	100.0	25.8bc	52.0
Prista 5	53.8c	0.0	36.2b	32.7	26.3b	51.1	0.00a	100.0	29.1b	46.0
Multifoliolate	60.1c	0.0	37.7b	37.3	35.7b	40.6	0.00a	100.0	33.4b	44.5
Roly	63.4c	0.0	37.7b	40.8	19.9a	68.8	0.00a	100.0	30.3b	52.4
Average	59.6c	0.0	35.2b	40.7	16.5a	70.8	0.00a	100.0		

a, b, c, d LSD at  $P=0.05$  confidence interval, GS<sub>%</sub> -germination seeds, IR -Inhibition rate

Seed germination was mostly unaffected by the tested fresh weed extract of *C. epithymum* only in the *M. sativa* variety „Multifoliolate“, while non-significant stimulating effects were only found at the highest concentrations (50 and 100 g/l<sup>-1</sup>). Similar results were reported by Othman et al. (2012), showing that fresh plant extracts of *C. campestris* did not reduce seed germination of radish (*R. sativus*).

This relationship could be due to allelochemicals (terpenes, long-chain fatty acids, phenols, phenolic acids and lactone) that are present in the parasitic weed species *C. campestris* (Khanh et al., 2008, Smith et al., 2016).

Allelochemicals are known to be toxic and have lethal effect on seeds at higher concentrations, whereas their lower concentrations inhibit seed germination of test plants (Macías et al., 2007; Yu et al., 2011; Othman et al., 2012; Baličević et al., 2014).

Depending on the type of extract (fresh or dry parasitic weed biomass), seed germination IRs of the tested alfalfa (*M. sativa*) varieties could be conventionally classified into five groups (Table 1): 1) seed germination stimulation

-1.4% – fresh biomass extracts of *C. epithymum* as affecting the variety „Multifoliolate“; 2) seed germination inhibition of 0.1-10 % – extracts prepared from fresh biomass of *C. epithymum* as affecting the varieties „Prista 3“, „Pleven 6“, „Prista 5“ and „Obnova“; 3) seed germination inhibition of 11- 20% – extracts of *C. epithymum* fresh biomass affecting the varieties „Roly“ and „Victoria“; 4) seed germination inhibition of 30-45% – extracts of dry biomass of *C. epithymum* affecting the variety „Multifoliolate“; 5) seed germination inhibition of 46-60% – extracts of *C. epithymum* dry biomass affecting the varieties „Prista 5“, „Prista 3“, „Victoria“, „Roly“, „Dara“, „Pleven 6“ and „Obnova“ (Table 1).

Differences in inhibitory effects of the extracts made from fresh or dry weed biomass of *C. epithymum* on seed germination of the test plants can be attributed to diffusion of soluble allelochemicals during extraction of fresh and dry weed biomass (Dhima et al., 2009; Konstantinović et al., 2014; Ravlić et al., 2016). Similar results were reported by Sisodia & Siddiqui (2010), Mondal et al. (2015), Petrova et al. (2015) and Ravlić

**Table 2.** Effects of different concentrations of aqueous extracts of *C. epithymum* on early seedling growth of *M. sativa* varieties, mm

Variety	Contents of weed biomass in water extracts, g l <sup>-1</sup>									
	0		25		50		100		Average	
	mm	IR	mm	IR	mm	IR	mm	IR	mm	IR
Fresh biomass										
Pleven 6	16.07b	0.0	16.30b	-1.4	14.92b	7.2	11.34a	29.4	14.66b	8.8
Dara	16.27c	0.0	14.83bc	8.9	12.77b	21.5	9.52a	41.5	13.35b	18.0
Obnova	14.67a	0.0	15.88a	-8.2	13.70a	6.6	14.31a	2.5	14.64a	0.2
Victoria	12.25a	0.0	10.91a	10.9	12.23a	0.2	12.09a	1.3	11.87a	3.1
Prista 3	16.77b	0.0	15.82b	5.7	15.63b	6.8	11.55a	31.1	14.94b	10.9
Prista 5	15.89b	0.0	14.63a	7.9	14.07a	11.5	14.17a	10.8	14.69a	7.6
Multifoliolate	17.87b	0.0	15.90a	11.0	13.77a	22.9	13.84a	22.6	15.35b	14.1
Roly	16.93b	0.0	16.00ab	5.5	15.81ab	6.6	13.00a	23.2	15.44ab	8.8
<i>Average</i>	15.84b	0.0	15.03b	5.0	14.11b	10.4	12.48a	20.3		
Dry biomass										
Pleven 6	16.07c	0.0	13.70c	14.7	8.33b	48.2	0.00a	100.0	9.53b	40.7
Dara	16.27b	0.0	14.50ab	10.9	8.50a	47.8	0.00a	100.0	9.82ab	39.7
Obnova	14.67c	0.0	12.33bc	16.0	3.33ab	77.3	0.00a	100.0	7.58a	48.3
Victoria	12.25b	0.0	14.00b	-14.3	10.50b	14.3	0.00a	100.0	9.19a	25.0
Prista 3	16.77c	0.0	13.71b	21.5	6.25ab	62.7	0.00a	100.0	9.18a	46.1
Prista 5	15.89c	0.0	15.36c	3.3	7.90b	50.3	0.00a	100.0	9.79a	38.4
Multifoliolate	17.87c	0.0	13.80b	26.9	12.00b	36.4	0.00a	100.0	10.92b	40.8
Roly	16.93b	0.0	10.87a	35.8	11.17a	34.0	0.00a	100.0	9.74b	42.5
<i>Average</i>	15.84c	0.0	13.53b	14.4b	8.50a	46.4	0.00a	100.0		

a. b. c. d. e LSD at P=0.05 confidence interval, IR -Inhibition rate

(2016) inferring that allelochemicals are released during extraction from fresh weed biomass, which does not occur during extraction from dry weed biomass. However, Qasem (1995) indicated that dry biomass may exhibit lower inhibitory potential than fresh biomass since the harmful effect could be greatly reduced during the drying process.

Similar results were obtained in our study determining the inhibition rate (IR) of seedling growth depending on the kind and content of weed biomass in water extracts (Table 2).

The rate of inhibition of seedling growth increased disproportionately with increase in weed biomass content in water extracts, from 0.2 to 18.0% on average for extracts from fresh weed biomass, and from 25.0 to 48.3% for extracts from dry weed biomass (Table 2). The mechanism of inhibition of seedling growth caused by allelochemicals can be the result of reduced cell division (Cheng & Cheng, 2015; Soltys et al., 2013).

There was a specific variety reaction with regard to the inhibitory effect of the extracts (fresh or dry parasitic

weed biomass) on seedling growth of the studied varieties of alfalfa (Table 2).

The applied concentrations of the aqueous extracts from dry and fresh biomass of *C. epithymum* had no statistically significant inhibitory effect on seedling growth of the *M. sativa* variety „Victoria“. A significant inhibitory effect of 14.1–40.8% of all applied concentrations (at  $P=0.05$ ) was found only in the variety „Multifoliolate“.

The extracts prepared from the fresh and dry parasitic weed biomass had various effects on the accumulation of fresh biomass in g per seedling of the test plants (Table 3). On average, the fresh biomass extracts of *C. epithymum* reduced accumulation of fresh biomass in g per seedling of alfalfa variety for up to 2.2%, while dry biomass extracts reduced the same parameter for over 50.0%, compared to controls.

The obtained experimental data confirmed the results of Seyyedi et al. (2013) and Nešić et al. (2016), according to which the impact of allelochemicals becomes evident already during seed germination but is even more pronounced during the subsequent growth of primary seedlings of plants.

**Table 3.** Effects of different concentrations of aqueous extracts of *C. epithymum* on early accumulation of fresh biomass (g) of seedlings of *M. sativa* varieties

Variety	Contents of weed biomass in water extracts, g l <sup>-1</sup>									
	0		25		50		100		Average	
	mm	IR	mm	IR	mm	IR	mm	IR	mm	IR
Fresh biomass										
Pleven 6	0.013a	0.00	0.012a	7.7	0.010a	23.1	0.009a	30.8	0.011a	15.4
Dara	0.013a	0.00	0.008a	32.5	0.010a	18.2	0.010a	20.0	0.010a	17.7
Obnova	0.010a	0.00	0.010a	0.0	0.010a	0.0	0.009a	10.0	0.010a	2.5
Victoria	0.014a	0.00	0.010a	26.8	0.008a	42.9	0.008a	42.9	0.010a	28.2
Prista 3	0.015a	0.00	0.013a	14.3	0.012a	24.3	0.012a	24.3	0.013a	15.7
Prista 5	0.012ab	0.00	0.013b	-8.3	0.013b	-8.3	0.009a	25.5	0.012ab	2.2
Multifoliolate	0.016a	0.00	0.016a	0.8	0.014a	15.2	0.013a	20.9	0.015a	9.2
Roly	0.014a	0.00	0.013a	8.8	0.010a	26.4	0.013a	5.5	0.013a	10.2
<i>Average</i>	0.013a	0.00	0.012a	10.3	0.011a	17.7	0.010a	22.5		
Dry biomass										
Pleven 6	0.013b	0.00	0.011b	15.4	0.001a	92.3	0.00a	100.0	0.006a	51.9
Dara	0.013b	0.00	0.010b	23.1	0.003a	76.9	0.00a	100.0	0.007ab	50.0
Obnova	0.010b	0.00	0.008b	20.0	0.001a	90.0	0.00a	100.0	0.005a	52.5
Victoria	0.014c	0.00	0.009b	35.7	0.002a	85.7	0.00a	100.0	0.006a	55.4
Prista 3	0.015c	0.00	0.011b	26.7	0.003a	80.0	0.00a	100.0	0.007ab	51.7
Prista 5	0.012b	0.00	0.011b	8.3	0.001a	91.7	0.00a	100.0	0.006ab	50.0
Multifoliolate	0.016b	0.00	0.015b	6.3	0.003a	81.3	0.00a	100.0	0.009ab	46.9
Roly	0.014c	0.00	0.008b	42.9	0.003a	78.6	0.00a	100.0	0.006b	55.4
<i>Average</i>	0.013c	0.00	0.010b	22.3	0.002a	84.6	0.00a	100.0		

a. b. c. d. e LSD at  $P=0.05$  confidence interval, IR -Inhibition rate

**Table 4.** Effects of different concentrations of aqueous extracts of *C. epithymum* on the initial development (GI) of *M. sativa* varieties

Variety	Contents of weed biomass in water extracts, g l <sup>-1</sup>							
	25	50	100	Average	25	50	100	Average
	Fresh biomass				Dry biomass			
Pleven 6	117.0	90.4	60.3	91.9	44.9	3.4	0.0	37.1
Dara	80.7	67.7	46.4	73.7	52.4	6.1	0.0	39.6
Obnova	101.7	93.4	77.7	93.2	41.3	2.8	0.0	36.0
Victoria	88.9	74.2	58.7	80.5	65.5	28.8	0.0	48.6
Prista 3	99.6	88.0	14.8	75.6	55.0	9.2	0.0	41.1
Prista 5	86.9	86.1	81.7	88.7	65.0	24.3	0.0	47.3
Multifoliolate	89.0	79.2	79.6	87.0	48.4	39.9	0.0	47.1
Roly	87.2	79.2	54.5	80.2	40.3	28.8	0.0	42.3
Average	93.9	82.3	59.2		51.6	17.9	0.0	

The index of germination (GI) depended on the same factors and followed the observed relationship pattern with regard to laboratory seed germination and growth of the seedlings of test plants (Table 4). The performed analyses showed that the studied extracts prepared from fresh and dry weed biomass provoked stimulating and/or inhibitory effects on the initial plant development of alfalfa varieties. With increasing weed biomass content in the water extracts made from fresh biomass of *C. epithymum*, GIs decreased 6.8-26.3% on average, while the decrease rate reached 64.0% in the variant of dry biomass extracts, as compared to control treatment. Exceptions to the described relationship were found only at 25 g l<sup>-1</sup> of extract from fresh biomass of *C. epithymum* in the alfalfa variety „Pleven 6“ (117.0%) and the variety „Victoria“ (101.7%).

Therefore, the observed differences among *M. sativa* varieties with regard to allelopathic effects of *C. epithymum* extracts may be attributed to the genetic differences of the examined alfalfa varieties because comparisons among them were performed at equal concentrations of extracts. Allelopathic tolerance was detected in the varieties „Victoria“, „Prista 5“ and „Multifoliolate“. Similar results in radish (*R. sativus*) and lettuce (*L. sativa*) were reported by Othman et al. (2012). They found that the species of test plants and their varieties had different levels of susceptibility to allelopathic effects of plant extracts (from fresh or dry biomass) and that they depended on the applied concentrations.

## CONCLUSION

The water extract prepared from dry biomass of *C. epithymum* showed a considerably stronger inhibitory effect on the studied *M. sativa* varieties (IR 32.7-100.0%) at P=0.05 statistical significance of difference, as compared to the extract of fresh biomass (IR from 0.2 to 40.5%).

Depending on the type of *C. epithymum* extract (fresh or dry parasitic weed biomass), the rates of inhibition of seed germination of the tested *M. sativa* varieties could be conventionally classified into five groups: the first group (seed germination stimulation -1.4%) includes extracts from fresh biomass as affecting the variety „Multifoliolate“; the second group (seed germination inhibition of 0.1- 10%) includes extracts prepared from fresh biomass as affecting the varieties „Prista 3“, „Pleven 6“, „Prista 5“ and „Obnova“; the third group (seed germination inhibition of 11-20%) includes extracts from fresh biomass as affecting the varieties „Roly“ and „Victoria“; the fourth group (seed germination inhibition of 30-45%) includes extracts from dry biomass as affecting the variety „Multifoliolate“; and the fifth group (seed germination inhibition of 46-60%) includes extracts from dry biomass as affecting the varieties „Prista 5“, „Prista 3“, „Victoria“, „Roly“, „Dara“, „Pleven 6“ and „Obnova“.

The studied alfalfa varieties showed different levels of susceptibility to the allelopathic effect of extracts prepared from fresh and dry biomass of *C. epithymum*, which was probably due to their genetic differences.

The alfalfa varieties „Victoria“, „Prista 5“ and „Multifoliolate“ possess some allelopathic tolerance because their index of germination (GI) ranged from 80.5 to 88.7% in extracts prepared from fresh weed biomass of *C. epithymum*, and from 47.1 to 48.6% in extracts from dry weed biomass, as compared to control treatments. These varieties can be used as components in future breeding programmes.

## REFERENCES

- Ahn, J.K., & Chung, I.M. (2000). Allelopathic potential of rice hulls on germination and seedling growth of barnyard grass. *Agronomy Journal*, 92, 1162-1167. doi:10.2134/agronj2000.9261162x
- Balićević, R., Ravlić, M., Knežević, M., Marić, K., & Mikić, I. (2014). Effect of marigold (*Calendula officinalis* L.) cogermination, extracts and residues on weed species hoary cress (*Cardaria draba* (L.) Desv.). *Herbologia*, 14(1), 23-32. doi 10.5644/Herb.14.1.03
- Bashir, U., Javaid, A., & Bajwa, R. (2011). Comparative tolerance of different rice varieties to sunflower phytotoxicity. *Journal of Medicinal Plants Research*, 5(26), 6243-6248. doi 10.5897/JMPR11.1143
- Bashir, U., Javaid, A., & Bajwa, R. (2012). Allelopathic effects of sunflower residue on growth of rice and subsequent wheat crop. *Chilean Journal of Agricultural Research*, 72(3), 326-331. doi.org/10.4067/S0718-58392012000300004.
- Bertholdsson, N.O., & Tuvesson, S. (2005). Possibilities to use marker assisted selection to improve allelopathic activity in cereals. In E.T. Lammerts van Bueren, I. Goldringer, H. Østergård (eds.), *Proceedings of the COST SUSVAR/ECO-PB Workshop on Organic Plant Breeding Strategies and the Use of Molecular Markers*, (pp 67-71). Driebergen, The Netherlands: Louis Bolk Institute. Retrieved from <http://www.louisbolk.org/downloads/1438.pdf>
- Cheng, F., & Cheng, Z., (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, 6, 1020. Retrieved from <https://doi.org/10.3389/fpls.2015.01020>
- Dhima, K., Vasilakoglou, I., Gatsis, T., Panou-Philotheou, E., & Eleftherohorinos, I. (2009). Effects of aromatic plants incorporated as green manure on weed and maize development. *Field Crops Research*, 110, 235-241. doi 10.1016/j.fcr.2008.09.005.
- Dimitrova, T. (2005). Proučavanje na plevelopodtiskashkata sposobnost na ljucernata, otglezhdana samostojatelno i v smeseni posevi (Study of weed inhibit the ability of alfalfa grown alone and in mixed crops). *Rasteniev" dni nauki (Plant Science)*, 42(5), 461-468.
- Dimitrova, T. (2008). A study of weed suppressive capacity of some cover crops as an alternative for weed control in lucerne (*Medicago sativa* L.). *Herbologia*, 9(1), 21-31. [http://www.anubih.ba/images/publikacije/herbologia/herbologia\\_09\\_1.pdf](http://www.anubih.ba/images/publikacije/herbologia/herbologia_09_1.pdf)
- Dimitrova, T., & Marinov-Serafimov, P. (2007a). Ecological approach against invasion of johnsongrass (*Sorghum halepense* (L.) Pers.) through mixed stands of lucerne with perennial grasses. *Herbologia*, 8(2), 13-20. Retrieved from [http://www.anubih.ba/images/publikacije/herbologia/herbologia\\_08\\_2.pdf](http://www.anubih.ba/images/publikacije/herbologia/herbologia_08_2.pdf)
- Dimitrova, T., & Serafimov, P. (2007b). Weed suppressive capacity of some perennial herbaceous mixtures – a possibility for nonchemical control of Canada thistle (*Cirsium arvense* L.). In A. De Vlieghe and L. Carlier (eds.), *Permanent and temporary grassland plant, environment and economy. Proceedings of the 14<sup>th</sup> Symposium of the European Grassland Federation* (vol. 12, pp 134-137), Ghent, Belgium: EGF. [http://www.europeangrassland.org/fileadmin/media/EGF2007\\_GSE\\_vol12.pdf](http://www.europeangrassland.org/fileadmin/media/EGF2007_GSE_vol12.pdf)
- Faravani, M., Baki, H., & Khalijah, A. (2008). Assessment of allelopathic potential of *Melastoma malabathricum* L. on radish *Raphanus sativus* L. and barnyard grass (*Echinochloa crus-galli*). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 36(2), 54-60. doi <http://dx.doi.org/10.15835/nbha36269>
- Fragasso M., Iannucci A., & Papa R. (2013). Durum wheat and allelopathy: Toward wheat breeding for natural weed management. *Frontiers in Plant Science*, 4, 368-375. doi <https://doi.org/10.3389/fpls.2013.00375>
- Gariglio, N.F., Buyatti, M.A., Pillati, R.A., Gozalez Russia, D.E., & Acosta, M.R. (2002). Use of a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. *New Zealand Journal of Crop and Horticultural Science*, 30, 135-139. Retrieved from <http://dx.doi.org/10.1080/01140671.2002.9514208>
- Hess, M., Barralis, G., Bleiholder, H., Buhr, L., Eggers, T. H., Hack, H., & Stauss, R. (1997). Use of the extended BBCH scale - general for the descriptions of the growth stages of mono; and dicotyledonous weed species. *Weed Research*, 37(6), 433-441. doi 10.1046/j.1365-3180.1997.d0170.x
- Hinkelman, K., & Kempthorne, O. (1994). *Design and analysis of experiments. Volume I: Introduction to experimental design* (p 495). New York, NY: John Wiley and Sons.
- ISTA (International Seed Testing Association). (1985). International rules for seed testing. *Seed Science and Technology*, 13, 361-513.
- Jabran, K., & Farooq, M. (2013). Implications of potential allelopathic crops in agricultural systems. In Zahid A. Cheema, Muhammad Farooq, Abdul Wahid (eds.), *Allelopathy* (pp 349-385). Berlin Heidelberg, Germany: Springer.

- Khanh, T.D., Cong, L.C., Xuan, T.D., Lee, S.J., Kong, D.S., & Chung, I.M. (2008). Weed-suppressing potential of dodder (*Cuscuta hygrophilae*) and its phytotoxic constituents. *Weed Science*, 56(1), 119-127. doi <http://dx.doi.org/10.1614/WS-07-102.1>
- Konstantinović, B., Blagojević, M., Konstantinović, B., & Samardžić, N. (2014). Allelopathic effect of weed species *Amaranthus retroflexus* L. on maize seed germination. *Romanian Agricultural Research*, 31, 315-321. Retrieved from <http://www.incda-fundulea.ro/rar/nr31/rar31.38.pdf>
- Macías, F.A., Molinillo, J.M.G., Varela, R.M., & Galindo, J.C.G. (2007). Allelopathy - A natural alternative for weed control. *Pest Management Science*, 63(4), 327-348. pmid:17348068. doi:10.1002/ps.1342
- Marinov-Serafimov, P., Dimitrova, Ts., Golubinova, I., & Ilieva, A. (2007). Study of suitability of some solutions in allelopathic researches. *Herbologia*, 8(1), 1-10. Retrieved from [http://www.anubih.ba/images/publikacije/herbologia/herbologia\\_08\\_1.pdf](http://www.anubih.ba/images/publikacije/herbologia/herbologia_08_1.pdf)
- Masum S.M., Hossain, M.A., Akamine, H., Sakagami, J.I., & Bhowmik, P.C. (2016). Allelopathic potential of indigenous Bangladeshi rice varieties. *Weed Biology and Management*, 16(3), 119-131. doi 10.1111/wbm.12103
- Miralles, P., Johnson, E., Church, T.L., & Harris, A.T. (2012). Multiwalled carbon nanotubes in alfalfa and wheat: Toxicology and uptake. *Journal of the Royal Society Interface*, 9(77), 3514-3527. doi 10.1098/rsif.2012.0535.
- Mondal, Md.F., Asaduzzaman, Md., & Asao, T. (2015). Adverse effects of allelopathy from legume crops and its possible avoidance. *American Journal of Plant Sciences*, 6(6), 804-810. doi 10.4236/ajps.2015.66086
- Nešić, M., Obratov-Petković, D., Skočajić, D., Bjedov, I., Đukić, M., & Đunisijević-Bojović, D. (2016). Allelopathic potential of the invasive species *Aster lanceolatus* Willd. *Periodicum biologorum*, 118(1), 1-7. doi <http://dx.doi.org/10.18054/pb.v118i1.2816>
- Okuno, K., & Ebana, K. (2003). Identification of QTL controlling allelopathic effects in rice: Genetic approaches to biological control of weeds. *Japan Agricultural Research Quarterly: JARQ*, 37(2), 77-81. doi <http://doi.org/10.6090/jarq.37.77>
- Othman, M.R., Leong, S.T., Bakar, B., Awang, K., & Annuar, M.S.M. (2012). Allelopathic potentials of *Cuscuta campestris* Yuncker extracts on germination and growth of radish (*Raphanus sativus* L.) and lettuce (*Lactuca sativa* L.). *Journal of Agricultural Science*, 4(9), 57-63. doi <http://dx.doi.org/10.5539/jas.v4n9p57>
- Petrova, S.T., Valcheva, E.G., & Velcheva, I.G. (2015). A case study of allelopathic effect on weeds in wheat. *Ecologia Balkanica*, 7(1), 121-129. Retrieved from [http://web.uni-plovdiv.bg/mollov/EB/2015\\_vol7\\_iss1/121-129\\_cb.15122.pdf](http://web.uni-plovdiv.bg/mollov/EB/2015_vol7_iss1/121-129_cb.15122.pdf)
- Qasem, J.R. (1995). Allelopathic effects of *Amaranthus retroflexus* and *Chenopodium murale* on vegetable crops. *Allelopathy Journal*, 2(1), 49-66. Retrieved from [http://www.allelopathyjournal.org/Journal\\_Articles/AJ%202%20\(1\)%20January,%201995%20\(49-66\).pdf](http://www.allelopathyjournal.org/Journal_Articles/AJ%202%20(1)%20January,%201995%20(49-66).pdf)
- Ravlić, M. (2016). Allelopathic effects of some plant species on growth and development of crops and weeds. *Poljoprivreda*, 22(1), 53-57. doi 10.18047/poljo.22.1.8
- Ravlić, M., Baličević, R., Nikolić, M., & Sarajlić, A. (2016). Assessment of allelopathic potential of fennel, rue and sage on weed species hoary cress (*Lepidium draba*). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 44(1), 48-52. doi <http://dx.doi.org/10.15835/nbha44110097>
- Rice, E.L. (1995). *Biological control of weeds and plant disease: Advances in applied allelopathy*. Norman, Oklahoma: University of Oklahoma Press.
- Seyyedi, M., Rezvani Moghaddam, P., Shahriari, R., Azad, M., & Eyshi Rezaei, E. (2013). Allelopathic potential of sunflower and castor bean on germination properties of dodder (*Cuscuta compestris*). *African Journal of Agricultural Research*, 8(7), 601-607. doi 10.5897/AJAR12.2136
- Sisodia, S., & Siddiqui, M.B. (2010). Allelopathic effect by aqueous extracts of different parts of *Croton bonplandianum* Baill. on some crop and weed plants. *Journal of Agricultural Extension and Rural Development*, 2(1), 022-028. Retrieved from <http://www.academicjournals.org/jaerd>
- Smith, J.D., Woldemariam, M.G., Mescher, M.C., Jander, G., & de Moraes, C.M. (2016). Glucosinolates from host plants influence growth of the parasitic plant *Cuscuta gronovii* and its susceptibility to aphid feeding. *Plant Physiology*, 172(1), 181-197. pmid:27482077
- Soltys, D., Gniazdowska, A., & Bogatek, R. (2013). Inhibition of tomato (*Solanum lycopersicum* L.) root growth by cyanamide is not always accompanied with enhancement of ROS production. *Plant Signaling & Behavior*, 8(5), e23994. doi 10.4161/psb.23994
- Trezzi, M.M., Vidal, R.A., Balbinot Jr, A.A., von Hertwig Bittencourt, H. & da Silva Souza Filho, A.P. (2016). Allelopathy: Driving mechanisms governing its activity in agriculture. *Journal of Plant Interactions*, 11(1), 53-60. doi 10.1080/17429145.2016.1159342
- Yu, H., Liu, J., He, W.M., Miao, S.L., & Dong, M. (2011). *Cuscuta australis* restrains three exotic invasive plants and benefits native species. *Biological Invasions*, 13(3), 747-756. doi 10.1007/s10530-010-9865-x



## Alelopatska otpornost više sorti lucerke (*Medicago sativa* L.) na vilinu kosicu (*Cuscuta epithymum* L.)

### REZIME

Ispitivano je alelopatsko delovanje hladnovodenih ekstrakta *Cuscuta epithymum* L. na klijanje semena i početni rast više različitih sorti *Medicago sativa* L. Ispitivanje je obavljeno u laboratorijskim uslovima u Institutu za krmno bilje, Pleven, Bugarska, tokom 2016-2017. Rezultati su pokazali da su vodeni rastvori suve mase *C. epithymum* imali značajno jače inhibitorno delovanje na proučavane sorte *M. sativa* (IR 32.7-100.0 %), u poređenju sa ekstraktima sveže mase (IR 0.2-40.5%). U odnosu na vrstu ekstrakta *C. epithymum* (sveža i/ili suva masa korova), stope inhibicije klijanja semena ispitivanih sorti *M. sativa* mogu se podeliti na pet grupa: 1) stimulisano klijanje semena, -1.4% – ekstrakti sveže mase u odnosu na sortu „Multifoliolate“; 2) klijanje semena inhibirano 0.1- 10% – ekstrakti sveže mase u odnosu na sorte „Prista 3“, „Pleven 6“, „Prista 5“ i „Obnova“; 3) klijanje semena inhibirano 11-20% – ekstrakti sveže mase u odnosu na sorte „Roly“ i „Victoria“; 4) klijanje semena inhibirano 30-45% – ekstrakti suve mase u odnosu na sortu „Multifoliolate“; 5) klijanje semena inhibirano 46-60% – ekstrakti suve mase u odnosu na sorte „Prista 5“, „Prista 3“, „Victoria“, „Roly“, „Dara“, „Pleven 6“ i „Obnova“.

Sorte lucerke „Victoria“, „Prista 5“ i „Multifoliolate“ poseduju alelopatsku otpornost pošto je njihov indeks klijanja (GI) bio u rasponu od 80.5 do 88.7 % u ekstraktima sveže mase *C. epithymum*, a od 47.1 do 48.6% u ekstraktima suve mase, u odnosu na kontrolu. Ove sorte se mogu koristiti kao komponente u razvoju budućih selekcionarskih programa.

**Keywords:** *Medicago sativa* L.; *Cuscuta epithymum* L.; Alelopatija; Klijanje semena; Inhibicija