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Contemporary Agriculture
Vol. 67, No. 1, Pp. 27 - 33, 2018.

The Serbian Journal of Agricultural Sciences
ISSN (Online) 2466-4774 UDC: 63(497.1)(051)-"540.2"
www.contagri.info

Original scientific paper
UDC: 633.31
DOI: 10.2478/contagri-2018-0004

ALLELOPATHIC EFFECT OF DODDER ON DIFFERENT VARIETIES OF LUCERNE AND BIRD'S FOOT-TREFOIL

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Summary: The aim of this study was to evaluate the allelopathic effect of cold aqueous extracts, both fresh and dry biomass of dodder (Cuscuta epithymum L.) on lucerne (Medicago sativa L.) and bird's foot-trefoil (Lotus corniculatus L.). Four different varieties of lucerne "Pleven 6", "Dara", "Roly", "Multifoliolate") and bird's foot-trefoil ("Gran San Gabriele", "Leo", "Local population 1", "Local population 2") were studied in order to find some varieties with allelopathic tolerance. Ex-situ experiment was carried out as follows: 100 seeds of each variety were put in Petri dishes between filter paper, both cold extracts of parasitic weed biomass were pipetted at a ratio of 1:20 as against the seed mass and then were placed in a thermostat-operated device at a temperature of 22 ± 2°C. Distilled water was used as a control. Percentage inhibition, Index of tolerance and Index of plant development were calculated for assessment of the allelopathic effect of dodder on the early seedling growth, biomass synthesis and initial development of experimental varieties. As a whole, dry weed biomass was found as more toxic for the tested plants than the fresh one. Medicago sativa var. multifoliolate and Lotus corniculatus var. Local population 1 and Local population 2 showed a significant tolerance to the allelopathic influence of Cuscuta epithymum in all studied concentrations of aqueous extract of fresh weed biomass (25, 50 and 100 g l^{-1}) and medium tolerance to aqueous extract of dry weed biomass (concentrations of 25 and 50 g l^{-1}).

Key words: allelopathy, Cuscuta epithymum, Medicago sativa, Lotus corniculatus

INTRODUCTION

Dodder control is economically very important in agricultural systems, where an annual drop of 10% in yield can be devastating. By debilitating the host plant, dodder decreases the ability of plants to resist viral diseases, and dodder can also spread plant diseases from one host to another if it is attached to more than one plant. Scientific researches in recent years have been focused mainly on creating resistant varieties and hybrids and on the development of highly effective systems for integrated weed control (Rubiales, 2012; Joel et al., 2013). There is a growing interest in allelopathy in agriculture at present, as this phenomenon could provide perspective alternative methods of weed management and help reduce the application of synthetic herbicides (Lopez-Raez, 2008).

The aim of this study was to evaluate the allelopathic effect of cold aqueous extracts, both fresh and dry biomass of dodder (*Cuscuta epithymum* L.) on lucerne (*Medicago sativa* L.) and bird's foot-trefoil (*Lotus corniculatus* L.). Previous studies have shown the allelopathic tolerance of some Bulgarian lucerne varieties ("Victoria", "Prista 5" and "Multifoliolate"), but there are no data concerning *L. corniculatus* (Marinov-Serafimov et al., 2017).

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MATERIAL AND METHODS OF THE STUDY

Collection and preparation of plant material:

Fresh biomass of dodder (*Cuscuta epithymum)* was collected in a natural environment of weed infestation in the region of the Institute of Forage Crops, Pleven, at the growth stage BBCH – 65-67 (Hess et al., 1997).

Seeds of 4 varieties of lucerne (*Medicago sativa* L.) and 4 varieties of *bird's foot-trefoil (Lotus corniculatus* L.) were taken from operational collections of the Institute of Forage Crops, Pleven and the Institute of Agriculture and Seed Science "Obraztsov Chiflik" – Ruse (Table 1).

Table. 1. Studied varieties of lucerne and bird's foot-trefoil

№	Species		Method of creation
	Scientific name	Genotypes	
1.	*Medicago sativa* L.	Pleven 6	variety
2.	*Medicago sativa* L.	Dara	variety
3.	*Medicago sativa* L.	Roly	variety
4.	*Medicago sativa* L.	Multifoliolate	variety
5.	*Lotus corniculatus* L.	Gran San Gabriele	variety
6.	*Lotus corniculatus* L.	Leo	variety
7.	*Lotus corniculatus* L.	Local population 1	Local population
8.	*Lotus corniculatus* L.	Local population 2	Local population

Plant extracts:

The ex-situ experiment was conducted under laboratory conditions at the Institute of Forage Crops in Pleven, Bulgaria, during 2015-2016. Aboveground biomass of *C. epithymum* was chopped to the length of 0.5-3.0 cm. Two kinds of weed extracts were prepared: A – from the fresh weed biomass from *C. epithymum*, crushed in advance with quartz sand and B – from dry weed biomass, after drying to a constant dry weight at 55 ± 2°C and grinding in grinder Retsch SM – 1 at a sieve size of 1.0 mm.

A hundred grams of dry and the same amount of fresh dodder biomass were soaked in 1 l distilled water and left for 24 h in a shuttle apparatus at 150/60 c^{-1} at a temperature of 24 ± 2°C. 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 in the text as g l^{-1}). Thymol ($C_{10}H_{14}O$) was added to each extract as a preserving agent (Marinov-Serafimov et al., 2007; Serafimov and Golubinova, 2015).

Bioassays:

A number of 100 seeds of lucerne and *bird's foot-trefoil* varieties were put in Petri dishes (diameter 90 mm) between filter paper. All available extracts, according to the parasitic weed biomass content, were pipetted at a ratio of 1:20 as against the seed mass (Marinov-Serafimov et al., 2007). Then, the samples were placed in a thermostat at 22 ± 2°C for seven days. Distilled water was used as a control. Each variant was laid out in ten replications.

Effect assessment:

Some qualitative and biometric parameters were used for assessing the results of the experiments: number of germinated seeds, length of the seedling (mm) and fresh biomass of the seedling (g). The length was measured using graph paper and the weight was recorded on an analytical balance.

Statistical evaluation of the allelopathic effect:

Germination seeds ($GS_{\%}$) was calculated according to ISTA (1985):

$$GS_{\%} = \frac{Number\ of\ seed\ germinated}{Total\ number\ of\ seed\ plated} \times 100$$

Percentage inhibition (I) was found according to the adapted formula of Surendra and Pota (1978):

$$I = 100 - \left(\frac{E_{treatment}}{E_{control}}\right) \times 100$$

where $E_{control}$ – measurement in the control treatment (seed germination and seedling biomass accumulation, g); $E_{treatment}$ - measurement in each treatment (seed germination and seedling biomass accumulation, g).

Tolerance Index (TI) was determined by the adapted formula of Tahseen and Jagannath (2015):

$$TI = \frac{LS_{TR}}{LS_{CT}} \times 100$$

where LS_{TR} – Longest of seedlings in each experimental treatment, mm;
LS_{CT} – Longest of seedlings in the control treatment, mm.

Index of plant development (*GI*) was assessed by the formula of Gariglio et al. (2002):

$$GI = \left[\left(\frac{G}{G_D}\right) \times \left(\frac{L}{L_D}\right)\right] \times 100$$

where *G* - germinated seeds in each treatment, %;

G_0 - germinated seeds in the control treatment, %;
L – average length (mm) of seedlings in the treatment transformed into percentage as against the control treatment;
L_0 – average length (mm) of the seedlings in the control treatment taken as 100%;
Percentage of seed germination in each treatment was previously transformed by the equation of Hinkelman and Kempthorne (1994):

$$Y = arcsin\sqrt{(\frac{X_{\%}}{100})}$$

where $x_{\%}$ – germinated seeds for each treatment (%).
All data were processed using the software packages Statgraphics + for Windows Ver. 2.1 and Statistica Ver. 10.

RESULTS

Aqueous extracts of fresh and dry dodder biomass showed an inhibitory effect on the seed germination of the studied plant species (Table 2). Dry weed biomass had a significantly stronger effect than the fresh one. Depending on the Percentage inhibition (I) due to the allelopathic influence of the maximum content of fresh weed biomass in aqueous extract (100 g l^{-1}), the studied varieties of lucerne and bird's foot-trefoil could be divided into 3 groups: (1) I<25% = Very tolerant – *M. sativa* var. Multifoliolate, *M. sativa* var. Pleven 6, *M. sativa* var. Dara, *L. corniculatus* var. Local population 1, *L. corniculatus* var. Leo; (2) I<50% = Tolerant - *M. sativa* var. Roly, *L. corniculatus* var. Local population 2; (3) I>75% = Very sensitive - *L. corniculatus* var. Gran San Gabriel. Regarding the allelopathic influence of the maximum content of dry weed biomass in aqueous extract (100 g l^{-1}), most of the studied varieties of lucerne and bird's foot-trefoil could be defined as very sensitive (I>75%), only *L. corniculatus* var. Local population 1 was found to be sensitive (I<75%). With the increase of weed biomass content, the germinated seed percentage decreased disproportionately in all treatments, as compared to the control variant, the differences being statistically significantly smaller at $P < 0.05$ (Table 2). Full doze of dodder biomass (100 g l^{-1}) was lethal for most varieties. Some exceptions were found for the lowest concentration of fresh biomass (25 g l^{-1}) where the effect was stimulating. Seed germination of *M. sativa* var. Multifoliolate was not inhibited by the fresh dodder biomass in all treatments and was medium inhibited (< 40%) by the dry dodder biomass at concentration 25 and 50 g l^{-1}. This fact revealed significant resistance of this variety to the allelopathic influence of the studied weed species.

Table 2. Effect of different concentrations of aqueous extracts of dodder (Cuscuta epithymum L.) on the process of seed germination

Species	Variety	Contents of the weed biomass in water extracts, g l^{-1}							
		0		25		50		100	
		GS%	*I*	*GS%*	*I*	*GS%*	*I*	*GS%*	*I*
		Fresh dodder biomass							
M. sativa	Pleven 6	56.8a	0.0	65.5b	-15.3	55.3a	2.6	48.5a	14.6
	Dara	71.6c	0.0	63.4b	11.5	61.8b	13.7	56.8a	20.7
	Multifoliolate	60.1a	0.0	60.1a	0.0	61.8a	-2.8	61.8a	-2.8
	Roly	63.4d	0.0	58.5c	7.7	53.8b	15.1	45.0a	29.0
L. corniculatus	Gran San Gabriele	47.9c	0.0	45.0bc	6.1	42.1b	12.1	0.0a	100.0
	Leo	53.8bc	0.0	55.3c	-2.8	49.4b	8.2	43.5a	19.1
	Local population 1	42.1ab	0.0	45.0b	-6.9	43.6ab	-3.6	40.7a	3.3
	Local population 2	40.0c	0.0	40.7c	-1.8	34.7b	13.3	28.3a	29.3
		Dry dodder biomass							
M. sativa	Pleven 6	56.8c	0.0	29.9b	47.4	3.7a	93.5	0.00a	100.0
	Dara	71.6c	0.0	42.1b	41.2	8.3a	88.4	0.00a	100.0
	Multifoliolate	60.1c	0.0	37.7b	37.3	35.7b	40.6	0.00a	100.0
	Roly	63.4c	0.0	37.7b	40.5	19.9a	68.6	0.00a	100.0
L. corniculatus	Gran San Gabriele	47.9c	0.0	29.9b	37.6	4.6a	90.4	0.0a	100.0
	Leo	53.8c	0.0	42.1b	21.7	6.7a	87.5	0.0a	100.0
	Local population 1	42.1b	0.0	42.0b	0.2	26.6a	36.8	15.0a	64.4
	Local population 2	40.0b	0.0	42.5b	-6.3	15.0a	62.5	7.5a	83.1

a, b, c, d – different letters in rows indicate significant statistical differences, LSD at P=0.05 confidence interval, $GS_{\%}$ -germination seeds, I - Percentage inhibition, %

Biometric measurements of the seedlings growth (mm) gave a possibility for objective estimation of the differences at the initial developmental stages of the studied lucerne and bird's foot-trefoil varieties depending on the type and the concentration of the applied dodder biomass (Table 3). Length of the seedlings was significantly influenced by the allelopathic parasitic plant and the inhibitory effect was stronger with the increment of the extract concentration ($P<0.05$), most pronounced at the dry dodder biomass. Some exceptions were found in the aqueous extract of 25 g l^{-1} fresh biomass where the seedlings in the experimental treatment were longer than the control - *M. sativa* var. Pleven 6, *L. corniculatus* var. Gran San Gabriel, *L. corniculatus* var. Leo.

Table 3. Effect of different concentrations of aqueous extracts of dodder (Cuscuta epithymum L.) on early seedling growth

Species	Variety	Contents of the weed biomass in water extracts, g l^{-1}							
		0		25		50		100	
		mm	*TI*	*mm*	*TI*	*mm*	*TI*	*mm*	*TI*
		Fresh dodder biomass							
M. sativa	Pleven 6	16.07b	100	16.30b	101.4	14.92b	92.8	11.34a	70.57
	Dara	16.27c	100	14.83bc	91.1	12.77b	78.5	9.52a	58.51
	Multifoliolate	17.87b	100	15.90a	89.0	13.77a	77.1	13.84a	77.45
	Roly	16.93b	100	16.00ab	94.5	15.81ab	93.4	13.00a	76.79
L. corniculatus	Gran San Gabriele	16.3b	100	17.0b	104.3	14.2b	87.1	0.0a	0.00
	Leo	21.9a	100	23.4a	106.8	21.8a	99.5	20.1a	91.78
	Local population 1	18.5a	100	16.5a	89.2	16.4a	88.6	13.4a	72.43
	Local population 2	18.6a	100	17.5a	94.1	16.1a	86.6	13.6a	73.12
		Dry dodder biomass							
M. sativa	Pleven 6	16.07c	100	13.70c	85.3	8.33b	51.8	0.00a	0.0
	Dara	16.27b	100	14.50ab	89.1	8.50a	52.2	0.00a	0.0
	Multifoliolate	17.87c	100	13.80b	77.2	12.00b	67.2	0.00a	0.0
	Roly	16.93b	100	10.87a	64.2	11.17a	66.0	0.00a	0.0
L. corniculatus	Gran San Gabriele	16.3b	100	17.2b	105.5	6.6a	40.5	0.0a	0.0
	Leo	21.9c	100	12.0b	54.8	8.8ab	40.2	0.0a	0.0
	Local population 1	18.5b	100	13.0a	70.3	10.4a	56.2	5.0a	27.03
	Local population 2	18.6a	100	15.6a	83.9	13.3a	71.5	9.7a	52.15

a, b, c, d – different letters in rows indicate significant statistical differences, LSD at P=0.05 confidence interval, $GS_{\%}$ -germination seeds, I - Percentage inhibition, %

Table 4. Effect of different concentrations of aqueous extracts of dodder (Cuscuta epithymum L.) on early seedling biomass accumulation

Species	Variety	Contents of the weed biomass in water extracts. g l^{-1}							
		0		25		50		100	
		g	*I*	*g*	*I*	*g*	*I*	*g*	*I*
		Fresh dodder biomass							
M. sativa	Pleven 6	0.013a	0.00	0.012a	7.7	0.010a	23.1	0.009a	30.8
	Dara	0.013a	0.00	0.008a	38.5	0.010a	23.1	0.010a	23.1
	Multifoliolate	0.016a	0.00	0.016a	0.0	0.014a	12.5	0.013a	18.8
	Roly	0.014a	0.00	0.013a	7.1	0.010a	28.6	0.013a	7.1
L. corniculatus	Gran San Gabriele	0.010c	0.000	0.008b	20.0	0.008b	20.0	0.000a	100.0
	Leo	0.011a	0.000	0.011a	0.0	0.010a	9.1	0.008a	27.3
	Local population 1	0.009a	0.000	0.011a	-22.2	0.009a	0.0	0.008a	11.1
	Local population 2	0.010a	0.000	0.008a	20.0	0.009a	10.0	0.008a	20.0
		Dry dodder biomass							
M. sativa	Pleven 6	0.013b	0.00	0.011b	15.4	0.001a	92.3	0.00a	100.0
	Dara	0.013b	0.00	0.010b	23.1	0.003a	76.9	0.00a	100.0
	Multifoliolate	0.016b	0.00	0.015b	6.3	0.003a	81.3	0.00a	100.0
	Roly	0.014c	0.00	0.008b	42.9	0.003a	78.6	0.00a	100.0
L. corniculatus	Gran San Gabriele	0.010b	0.000	0.011b	-10.0	0.001a	90.0	0.000a	100.0
	Leo	0.011c	0.000	0.007b	36.4	0.001a	90.9	0.000a	100.0
	Local population 1	0.009b	0.000	0.009b	0.0	0.007b	22.2	0.001a	88.9
	Local population 2	0.010b	0.000	0.005ab	50.0	0.002a	80.0	0.002a	80.0

a, b, c, d – different letters in rows indicate significant statistical differences, LSD at P=0.05 confidence interval, $GS_{\%}$ -germination seeds, I - Percentage inhibition, %

According to the Tolerance index (TI) values from the fresh dodder biomass effect (100 g l^{-1}), most of the studied varieties could be classified as very tolerant (TI>75%) and tolerant (TI>50%), only *L. corniculatus* var. Gran San Gabriel had no germinated seeds. In the case of the Tolerance index (TI) of the dry dodder biomass, we used the medium concentration of 50 g l^{-1}, as the highest dose was almost equal to LC_{100}. The results divided the studied forage varieties into 2 groups: (1) TI>50% = Tolerant - *M. sativa* var. Roly, *M. sativa* var. Pleven 6, *M. sativa* var. Dara, *M. sativa* var. Multifoliolate, *L. corniculatus* var. Local population 1, *L. corniculatus* var. Local population 2; (2) TI>25% = Sensitive - *L. corniculatus* var. Gran San Gabriel, *L. corniculatus* var. Leo.

Fresh biomass synthesis was also inhibited at a different degree depending on the type of weed biomass and the applied concentration (Table 4). *L. corniculatus* var. Local population 1 was found to be less sensitive to the fresh dodder biomass extract, as at 25 g l^{-1} a stimulating effect was found, at 50 g l^{-1} the effect was neutral and at 100 g l^{-1} the percentage of inhibition was only 11.1%. This variety showed also a high tolerance to the allelopathic effect of dry dodder biomass in 25 g l^{-1} and 50 g l^{-1} concentrations. The other experimental forage varieties exhibited a light to medium negative influence on the biomass accumulation due to the fresh weed biomass extract and a very strong negative influence due to the dry weed biomass extract.

Index of initial plant development (GI) depended on both seed germination and seedling growth, thus followed the above mentioned relationship patterns (Table 5). Stimulation was observed in the initial development of *M. sativa* var. Pleven 6 (117%) and *L. corniculatus* var. Leo (109.8%) under 25 g l^{-1} fresh dodder biomass extract and of *L. corniculatus* var. Gran San Gabriele (107.4%) under 25 g l^{-1} dry dodder biomass extract. Maximum tolerance to the allelopathic effect of both fresh and dry weed biomass in the higher concentrations was found for *M. sativa* var. Multifoliolate and *L. corniculatus* var. Local population 1.

Table 5. Effect of different concentrations of aqueous extracts of dodder (Cuscuta epithymum L.) on the initial development (GI) of the seedlings

Species	Variety	Contents of the weed biomass in water extracts. g l^{-1}					
		25	50	100	25	50	100
		Fresh dodder biomass			Dry dodder biomass		
M. sativa	Pleven 6	117.0	90.4	60.3	44.9	3.4	0.0
	Dara	80.7	67.7	46.4	52.4	6.1	0.0
	Multifoliolate	89.0	79.2	79.6	48.4	39.9	0.0
	Roly	87.2	79.2	54.5	40.3	28.8	0.0
L. corniculatus	Gran San Gabriele	98	76.6	0.0	107.4	35.1	0.0
	Leo	109.8	91.4	74.2	42.9	5.0	0.0
	Local population 1	77.6	79.3	64.8	70.1	35.5	9.6
	Local population 2	95.7	75.1	51.7	89.1	26.8	9.8

DISCUSSION

Seed germination is a critical phase in the life cycle of most plant species, especially the cultural ones. Unfavorable conditions of the environment have a strong impact on the germination processes. Roots of the plants first come into contact with the environmental pollutants and the suppression of the growth is a consequence of that of the root as it limits the intake of water and mineral substances, and also because of the direct effect of inhibitors on the cellular metabolism (Vince and Zoltan, 2011).

In the present study we found that the aqueous extract of 100 g l^{-1} dry dodder biomass had a lethal effect on the seed of all four studied varieties of lucerne and on the seeds of two varieties of bird's foot-trefoil (Table 2). Only seeds of *L. corniculatus* var. Local population 1 and *L. corniculatus* var. Local population 2, which have been collected from naturally growing populations, had germinated (35.6% and 16.9%, respectively). Initial development of the seedlings in these two experimental variants was significantly inhibited, as follows: growth – by 72.9% and 48.1%; biomass accumulation – by 88.9% and 80%; plant development – by 90.4% and 90.2%. Aqueous extracts of the two lower concentrations of dry dodder biomass had also an inhibitory effect on seed germination and seedling development but it was not so heavily expressed (Table 2, 3, 4 and 5). Marinov-Serafimov et al. (2017) have reported that LC_{50} for seed germination of the test plant *Lactuca sativa* is in the range 1.91-2.60% w/v dry biomass of *Cuscuta epithymum*, which corresponds to our findings for the most of the studied lucerne and bird's foot-trefoil varieties, except for *L. corniculatus* var. Local population 1 and *M. sativa* var. Multifoliolate.

This relationship could be explained by the presence of glycol alkaloids and condensed tannins in aboveground weed biomass. It is well known that these two groups of chemical substances exert a strong toxicity, so at higher concentration they have a lethal effect, whereas at lower concentrations they inhibit the seed germination to a different extent (Agarwal et al., 2002). Differences in the negative effect on the germination process could be due also to the diffusion of the soluble allelochemicals from the dodder biomass into the agar (Sangeetha and Baskar, 2015). Similar results were reported by Fernández-Aparicio et al. (2006), Qasem (2010), Lev-Yadun (2013), according to whom allelopathic effect was species specific and depended on the applied concentrations. The data obtained corresponded with the findings of Ashrafi et al. (2007) and Othman (2012) that the effect of the allelochemicals is manifested already during the seed germination, but it is more pronounced during the growth of primary seedlings of plants.

CONCLUSION

As a whole, dry weed biomass was found as more toxic for the tested plants than the fresh one. *Medicago sativa* var. Multifoliolate and *Lotus corniculatus* var. Local population 1 and Local population 2 showed a significant tolerance to the allelopathic influence of *Cuscuta epithymum* in all studied concentrations of aqueous extract of fresh weed biomass (25, 50 and 100 g l^{-1}) and medium tolerance to aqueous extract of dry weed biomass (concentrations of 25 and 50 g l^{-1}). The experiment should be continued in field conditions using the above mentioned varieties in order to track the allelopathic effect of *Cuscuta epithymum* on their development throughout the whole vegetation cycle.

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Received / Primljen: 12.06.2017.
Accepted / Prihvaćen:18.12.2017.