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Allelopathic effect of dodder *(Cuscuta epithymum* L.) on different genotypes bird's-foot trefoil (*Lotus corniculatus* L.)

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Abstract

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During the 2015-2016 period the allelopathic effect of cold water extracts from *Cuscuta epithymum* L. on seed germination and initial development of *Lotus corniculatus* L. genotypes were studied under laboratory conditions in the Institute of Forage Crops, Pleven. It was found that: different aqueous extract concentrations of parasitic plant directly influenced germination percentage, rate and seedling length of *L. corniculatus* genotypes; the water extracts from dry biomass of *Cuscuta epithymum* L. showed a considerably stronger inhibitory effect on the studied *Lotus corniculatus* L. genotypes on growth seedlings ($I_{(average)}$ from 32.7 to 89.1) where the differences are statistically significant at P = 0.05, as compared to the extract of fresh biomass ($I_{(average)}$ from 1.8 to 17.6). The studied *Lotus corniculatus* L. genotypes showed different susceptibility to the allelopathic effect of the extracts from the extracts of fresh and dry biomass of *Cuscuta epithymum* L., which was due to their genetic differences. *Lotus corniculatus* L. genotypes Stamm 02, Local population 1 and Local population 2 possess allelopathic potential, because index germinations (GI) were in the range from 51.7 to 95.7% in extracts prepared from fresh weed biomass of *Cuscuta epithymum* L. and from 26.8 to 9.6% to dry weed biomass. These genotypes can be used as components in future breeding programmes.

Keywords: Cuscuta epithymum; *Lotus corniculatus*; seed germination; inhibition; allelopathic tolerance

Introduction

The significant progress in the study of allelopathic interaction between plants is a prerequisite allelopathy and is becoming widely recognized as a scientific discipline. Allelopathy is a sub-discipline of chemical ecology that is concerned with the effects of chemicals produced by plants or microorganisms on the growth, development and distribution of other plants and microorganisms in natural communities or agricultural systems (Einhellig, 1995; Inderjit et al., 2005). Allelochemicals are plant metabolites or their products that are released into the environment and rhizosphere through volatilization, exudation from roots, leaching from plants or residues, and decomposition of residues (Rice, 1984; Iannucci et al., 2013). Allelochemicals probably are natural defenses for plants against disease, insects, parasites, or herbivores (War et al., 2012; Fürstenberg-Hägg et al., 2013).

Parasitic weeds represent a main component of weed problems facing agriculture and negatively impact agroecosystem and environment (Qasem, 2006). Parasitic weeds decrease severely the production of major grain and forage legumes (Rubiales & Fernández-Aparicio, 2012). They are highly ecological, biological and physiological tolerant species to both internal and external environmental changes and relationship between these parasites and their hosts is highly complicated (Qasem, 2006). There is no single technology to control these parasitic weeds (Parker, 2009; Rubiales et al., 2009). Easy infestation with parasitic weed of plants is because of huge production of viable seeds. Parasitic weeds germinate only in response to specific chemicals released by the host plant. Screening tests have shown a wide range of allelopathic activity in different crops (Belz, 2007; Zhou et al., 2011). Even among different cultivars of the one crop, some genotypes may have substantial genetically determined allelopathic activity (Baghestani et al., 1999). One of the methods of weed management strategy is the detection of genotypes with high allopathic potential and use in breeding programs. This is ecological and economical profitable. The possibility of use of genotypes with an allopathic potential would reduce the need for applying herbicides to the crop, is worth exploring (Khush, 1996; Jabran et al., 2013).

The common bird's-foot trefoil (*Lotus corniculatus* L.) is important legume fodder grass species, with large potential, the high ecological plasticity, with a wide variety of cultivars (Widdup et al., 2004; Chapman et al., 2008; Churkova, 2015; Vasileva & Ilieva, 2016). The dynamics and hight level weed infestation in grasslands of forage crops are a prerequisite for decreasing yields (MacLean & Grant, 2011; Churkova & Bozhanska, 2016a, 2016b). In Bulgaria hed sporadik results have been published about allelopathic tolerance of bird's-foot trefoil (*Lotus corniculatus* L.) genotypes to different weed species (Valcheva et al., 2018).

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 bird's-foot trefoil (*Lotus corniculatus* L.) genotypes, and to identify the genotypes with allelopathic tolerance of that parasitic weed.

The objective of this study was to determine allelopathic effect of aqueous extracts, fresh and dry biomass of dodder (*Cuscuta epithymum* L.) on seed germination, growth and development of seedlings in different genotypes of bird's-foot trefoil (*Lotus corniculatus* L.), and detection of genotypes with allelopathic tolerance.

Material and Methods

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

Collection and Preparation of Plant Material:

The seeds of bird's-foot trefoil *(Lotus corniculatus* L.) by different varieties (Table 1) were taken from operational collections of the Institute of Forage Crops, Pleven.

The biomass from of *C. 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).

Plant extracts: 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 biomass from the *C. epithymum* were soaked in $1/l^{-1}$ distilled water. The samples prepared in such way that fresh or dry biomass of *C. epithymum* were cold extracted at a temperature of 24±2°C for 24 h in a shuttle apparatus at $150/60\ c^{-1}$. 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 & Golubinova, 2017).

Bioassays: A number of 100 seeds of bird's-foot trefoil *(L. corniculatus*) genotypes were put in the 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). Distilled water was used as a control. Each variant was laid out in ten replications. The samples

Table 1. Variety of bird's-foot trefoil (*Lotus corniculatus* L.)

№	Genotypes		Method of creation	Country
	Scientific name	*Common name*		
1.	*Lotus corniculatus* L.	Targovishte	Variety	Bulgaria
2.	*Lotus corniculatus* L.	Gran San Gabriele	Variety	Italy
3.	*Lotus corniculatus* L.	Leo	Variety	Canada
4.	*Lotus corniculatus* L.	Taborac	Variety	Hungary
5.	*Lotus corniculatus* L.	Stamm 1	Local population	Austria
6.	*Lotus corniculatus* L.	Stamm 2	Local population	Austria
7.	*Lotus corniculatus* L.	Local population 1	Local population	Bulgaria
8.	*Lotus corniculatus* L.	Local population 2	Local population	Bulgaria

were then placed in a thermostat-operated device at a temperature of 22 ± 2°C for seven days.

Effect assessment: The following biometric parameters were used for assessing the results of the experiments: length of the seedling (mm) and fresh biomass of the seedling, (g). Length was measured using graph paper and the weight was recorded on an analytical balance.

Statistical evaluation and calculated formulas: Germination seeds ($GS_{\%}$) was determined by the Equation (1) prescribed according to ISTA (1985):

$$GS\% = \frac{\textit{Number of seed germinated}}{\textit{Total number of seed planted}}.100 \quad (1)$$

The percentage inhibition (*I*) was determined an adapted formula by Surendra and Pota (1978)**,** Equation (2):

$$I = 100 - \frac{(E_2.100)}{E_1} \quad (2)$$

where E_1 – measurement of the control treatment; E_2 – measurement of in each treatment, mm;

The index of plant development (*GI*) was determined by the Equation (3) (Gariglio et al., 2002):

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

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 the 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 analysed using the software Statgraphics Plus for Windows Ver. 2.1 and STATISTICA Ver. 10.

Results and Discussion

The effect of aqueous extracts of *C. epithymum* was evaluated on the germination of seeds and seedling growth of different cultivars of *L. corniculatus*. The concentrations of the applied extracts of *C. epithymum* had a substantial influence on laboratory germination of seed the studied genotypes (Table 2). With increasing aqueous extract (fresh and dry biomass) concentration there was a general tendency of germination decrease average in all genotypes, as against the control treatment (Table 2). 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 $P < 0.05$.

Germination seeds in *L. corniculatus* genotypes Leo and Local population 1, was mostly unaffected by the tested extract of parasitic fresh weed biomass of *C. epithymum* while no significant stimulant effect was found for the highest concentrations (50 and 100 g/l^{-1}).

Similar results were reported by Othman et al. (2012) according to them the fresh plant extracts of *C. campestris* did not reduce seed germination of radish (*Raphanus sativus*).

This relationship could be explained by the presence of allelochemicals (terpenes, long-chain fatty acids, phenols, phenolic acids, and lactone) in the *C. campestris* parasitic weed species (Khanh et al., 2008, Smith et al., 2016).

The dry biomass extract of *C. epithymum* showed a considerably average stronger inhibitory effect on the all studied bird's foot-trefoil genotypes (*I* from 26.0 to 93.2%) and the differences are statistically significant at P=0.05, as compared to the extract of fresh biomass (*I* from 2.4 to 22.9%). The extract of dry biomass only in lowest concentration (25 g/l^{-1}) has stimulating effect at the Targovishte, Gran San Gabriel, Leo and genotypes, but the differences were not statistically significant at the P=0.05. The inhibitory effect on germination of seeds of *L. corniculatus* probably due to the considerably higher concentrations of extract, compared to concentrations in agrophytocenoses.

Effects of fresh biomass fom aqueous extracts of *Cuscuta epithymum* on shoot length and seedling biomass of *L. corniculatus* genotypes were insignificant (Table 3). Extract of fresh biomass in the all concentrations did not considerably reduce seedling length of *L. corniculatus* genotypes and the differences from untreated control are not significant at P=0.05. Only at the lowest dose (25 g/l^{-1}) there is a slight stimulating effect (*I* from 1.1 to 6.8) on the of *L. corniculatus* genotypes Targovishte, Gran San Gabriel, Leo and Taborac. Uppermost decrease in seedling length average for all genotypes was obtained under the highest (100 g/l^{-1}) extract concentration (*I* is 17.6%).

The present study revealed strong allelopathic effects of dry biomass of *C. epithymum* on *L. corniculatus* genotypes on germination and seedling growth parameters.

The shoot length was significantly reduced by all concentrations of all dry biomass aqueous extracts where was average from 32.1 at 25 g/l^{-1} to 89.1% at the highest dose of 100 g/l^{-1} and the differences are significant according to control

Table 2. Effect of different concentrations of aqueous extracts of *C. epithymum* on germination of seeds of *L. corniculatus* genotypes, %

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 biomass							
Targovishte	47.9c	0.0	45.0bc	6.1	42.1b	12.1	38.0a	20.7
Gran San Gabriel	53.8bc	0.0	55.3c	-2.8	49.4b	8.2	43.5a	19.1
Leo	43.6a	0.0	50.8b	-16.5	43.6a	0.0	45.0a	-3.2
Taborac	45.0c	0.0	34.7ab	22.9	33.2a	26.2	37.7b	16.2
Stamm 01	56.8c	0.0	53.8bc	5.3	49.4a	13.0	52.3ab	7.9
Stamm 02	56.8b	0.0	49.4a	13.0	50.8a	10.6	50.8a	10.6
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
Average	48.3	0.0	46.8	2.4	43.4	10.0	37.3	22.9
	Dry biomass							
Targovishte	47.9c	0.0	29.9b	37.6	4.6a	90.4	0.0a	100.0
Gran San Gabriel	53.8c	0.0	42.1b	21.7	6.7a	87.5	0.0a	100.0
Leo	43.6b	0.0	40.7b	6.7	0.0a	100.0	0.0a	100.0
Taborac	45.0b	0.0	9.8a	78.2	0.0a	100.0	0.0a	100.0
Stamm 01	56.8c	0.0	39.2b	31.0	9.8a	82.7	0.0a	100.0
Stamm 02	56.8c	0.0	34.7b	38.9	31.4b	44.7	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	81.3
Average	48.3	0.0	35.1	26.0	24.4	75.6	2.8	93.2

Legend: Means with different letters differ at $P < 0.05$ level of probability by LSD test, $GS_{\%}$ – germination seeds, I – percentage inhibition, %

Table 3. Effect of different concentrations of aqueous extracts of *C. epithymum* on early seedling growth of *L. corniculatus* genotypes, mm

Variety	Contents of the weed biomass in water extracts, g/l^{-1}							
	0		25		50		100	
	mm	I	mm	I	mm	I	mm	I
	Fresh biomass							
Targovishte	16.3a	0	17.0b	-4.3	14.2b	12.9	12.9a	20.9
Gran San Gabriel	21.9bc	0	23.4ba	-6.8	21.8a	0.5	20.1a	8.2
Leo	21.2a	0	22.1a	-4.2	21.2a	0.0	20.1a	5.2
Taborac	17.7ab	0	17.9b	-1.1	17.5b	1.1	11.1a	37.3
Stamm 01	23.1c	0	20.1a	13.0	21.9a	5.2	20.4a	11.7
Stamm 02	17.0a	0	16.8a	1.2	16.9a	0.6	16.5a	2.9
Local population 1	18.5abc	0	16.5a	10.8	16.4a	11.4	13.4a	27.6
Local population 2	18.6abc	0	17.5a	5.9	16.1a	13.4	13.6a	26.9
Average	*19.3*	0	*18.9*	*1.8*	*18.3*	*5.6*	*16.0*	*17.6*
	Dry biomass							
Targovishte	16.3b	0	17.2b	-5.52	6.6a	59.5	0.0a	100.0
Gran San Gabriel	21.9c	0	12.0b	45.2	8.8ab	59.8	0.0a	100.0
Leo	21.2c	0	12.1b	42.9	0.0a	100.0	0.0a	100.0
Taborac	17.7b	0	4.8a	72.9	0.0a	100.0	0.0a	100.0
Stamm 01	23.1c	0	10.9b	52.8	6.0ab	74.0	0.0a	100.0
Stamm 02	17.0b	0	18.8b	-10.6	14.2b	16.5	0.0a	100.0
Local population 1	18.5b	0	13.0a	29.7	10.4a	43.8	5.0a	73.0
Local population 2	18.6a	0	15.6a	16.1	13.3a	28.5	9.7a	47.8
Average	*19.3*	0	*13.1*	32.1	*7.4*	61.7	*2.1*	89.1

Legend: Means with different letters differ at $P < 0.05$ level of probability by LSD test, I – percentage inhibition, %

treatments. Therefore, the extract from dry biomass from *C. epithymum* showed a cconsiderably stronger inhibitory effect on the studied *L. corniculatus* genotypes (*I* from -10.6 to 100.0) where the differences are statistically significant at P = 0.05, as compared to the extract of fresh biomass (*I* from -10.6 to 100.0) (Table 3).

A strong negative correlation was discovered between the seedling growth of the *L. corniculatus* genotypes and extracts from dry biomass froom *C. epithymum* where *r* varied between 0.900 and 0.981 and from weak (r = -0.128) to strong (r = -0.995) negative correlations depending on the inhibitory effect of fresh parasitic weed biomass. That was probably due to considerably higher used concentrations of the extract, as compared to those found in the agrophytocenoses with falling off and decomposition of weed biomass.

The exception is found for the extract of fresh weed biomass on *C. epithymum* only in genotype Stamm 02, was mostly unaffected by the tested extract of parasitic dray weed biomass of *C. epithymum* which establishes an stimulation effect (from 10.6 %), and the differences were statistically significant at the P = 0.05 (Table 3).

The differences inhibitory effect of the extracts from fresh or dry weed biomass can be explained by diffusion of soluble allelochemicals during extraction (Hussain et al., 2014; Khan et al., 2015). Probably, during extraction from dray weed biomass the allelochemicals are released, which does not occur during extraction from fresh weed biomass.

The accumulation of fresh biomass in g per seedlings of the development of the tested of *L. corniculatus* genotypes depends on the same factors and the observed dependencies from the growth of the seedlings, mm depending on the application of fresh or dry parasitic weed biomass (Table 4).

The index germinations (GI) depended on the same factors and followed the observed relationship pattern with regard to laboratory seed germination and growth of seedling of test *L. corniculatus* genotypes (Table 4).

The performed analyses showed that the studied extracts from fresh and dry weed biomass provoked a suppressive or inhibitory effect on the plant development of bird's-foot trefoil genotypes. With increase of the weed biomass content in the water extracts from fresh biomass from *C. epithymum,* GI decreased average from 4.30 to 35.7% and for the extracts from dry biomass this decrease rate reached 97.6% for 100 g/l parasitic weed biomass, as compared to the control treatment.

Table 4. Effect of different concentrations of aqueous extracts of *C. epithymum* on early accumulation fresh biomass (g) of the seedlings of *L. corniculatus* genotypes

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 biomass							
Targovishte	0.010c	0.0	0.008b	20.0	0.008b	20.0	0.008a	20.0
Gran San Gabriel	0.011a	0.0	0.011a	0.0	0.010a	9.1	0.008a	27.3
Leo	0.010ab	0.0	0.012b	-20.0	0.009ab	10.0	0.008a	20.0
Taborac	0.009a	0.0	0.007a	22.2	0.008a	11.1	0.007a	22.2
Stamm 01	0.010a	0.0	0.010a	0.0	0.011a	-10.0	0.010a	0.0
Stamm 02	0.013a	0.0	0.013a	0.0	0.011a	15.4	0.010a	23.1
Local population 1	0.009a	0.0	0.011a	-22.2	0.009a	0.0	0.008a	11.1
Local population 2	0.010a	0.0	0.008a	20.0	0.009a	10.0	0.008a	20.0
Average	0.010	0.0	0.010	2.5	0.009	8.2	0.008	18.0
	Dry biomass							
Targovishte	0.010b	0.0	0.011b	-10.0	0.001a	90.0	0.000a	100.0
Gran San Gabriel	0.011c	0.0	0.007b	36.4	0.001a	90.9	0.000a	100.0
Leo	0.010b	0.0	0.010b	0.0	0.000a	100.0	0.000a	100.0
Taborac	0.009b	0.0	0.001a	88.9	0.000a	100.0	0.000a	100.0
Stamm 01	0.010c	0.0	0.005b	50.0	0.001a	90.0	0.000a	100.0
Stamm 02	0.013c	0.0	0.006b	53.8	0.006b	53.8	0.000a	100.0
Local population 1	0.009b	0.0	0.009b	0.0	0.007b	22.2	0.001a	88.9
Local population 2	0.010b	0.0	0.005ab	50.0	0.002a	80.0	0.002a	80.0
Average	0.010	0.0	0.007	33.64	0.002	78.4	0.000	96.1

Legend: Means with different letters differ at P < 0.05 level of probability by LSD test, I – percentage inhibition, %

Table 5. Effect of different concentrations of aqueous extracts of *C. epithymum* on the initial development (GI) *L. corniculatus* genotypes

Variety	Contents of the weed biomass in water extracts, g/l^{-1}					
	25	50	100	25	50	100
	Fresh biomass			Dry biomass		
Targovishte	98.0	76.6	0.0	65.9	3.9	0.0
Gran San Gabriel	109.8	91.4	74.2	42.9	5.0	0.0
Leo	121.5	100.0	97.9	53.3	0.0	0.0
Taborac	78.0	72.9	52.5	5.9	0.0	0.0
Stamm 01	82.4	82.5	81.3	32.6	4.5	0.0
Stamm 02	85.9	88.9	86.8	67.6	46.2	0.0
Local population 1	95.3	91.8	70.0	70.1	35.5	9.6
Local population 2	95.7	75.1	51.7	89.1	26.8	9.8
Average	95.8	84.9	64.3	53.4	15.2	2.4

An exception to the described relationship was found only at 25 g/l for extracts from fresh biomass of *C. epithymum* in bird's foot-trefoil variety Leo (125.1%). Therefore, the observed differences in *L. corniculatus* genotypes with regard to allelopathic effect of the extracts from *C. epithymum* can be explained by genetic differences, because the comparisons between them were performed at equal concentrations of the applied extracts, which determined the presence of allelopathic tolerance in genotypes Stamm 02, Local population 1 and Local population 2. Similar results in radish (*Raphanus sativus* L.) and lettuce (*Lactuca sativa* L.) were reported by Othman et al. (2012), according to whom the species of test plants and genotypes had different susceptibility to allelopathic effect of plant extracts (from fresh or dry biomass) and depended on the applied concentrations.

Conclusions

The results showed that different aqueous extract concentrations of parasitic plant directly influenced germination percentage, rate and seedling length of *L. corniculatus* genotypes.

The water extracts from dry biomass of *Cuscuta epithymum* L. showed a considerably stronger inhibitory effect on the studied *Lotus corniculatus* L. genotypes on growth seedlings ($I_{(average)}$ from 32.7 to 89.1) where the differences are statistically significant at P = 0.05, as compared to the extract of fresh biomass ($I_{(average)}$ from 1.8 to 17.6).

Lotus corniculatus L. genotypes showed different susceptibility to the allelopathic effect of the extracts from of extracts of fresh and dry biomass of *Cuscuta epithymum* L., which was due to their genetic differences.

Lotus corniculatus L. genotypes Stamm 02, Local population 1 and Local population 2 possess allelopathic potential, because index germinations (GI) in the range from 51.7 to 95.7% in extracts prepared from fresh weed biomass of *Cuscuta epithymum* L. and from 9.6 to 89.1% to dry weed biomass. These genotypes can be used as components in future breeding programmes.

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