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Allelopathic activity of rhizosphere soil in alfalfa - *Sorghum* sp. mixed growing

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ABSTRACT

The allelopathic activity of soil from rhizosphere zone of alfalfa (*Medicago sativa* L.) grown under cover of *Sorghum bicolor* L. *Sorghum sudanense* Piper Staf. and *Sorghum vulgare* var. *technicum* Korn. on the initial development of *Lactuca sativa* L. was assessed. The experiment was performed under laboratory conditions at the Institute of Forage Crops - Pleven, Bulgaria (2018-19). The highest inhibition effect showed the soil from the rhizosphere zone of *Sorghum vulgare* var. *technicum* (17.44) pure grown; alfalfa, grown under cover of *Sorghum vulgare* var. *technicum* (14.56) and alfalfa, grown under cover of *Sorghum bicolor* + *Sorghum vulgare* var. *technicum* (16.94). The lowest inhibition effect showed the soil from the alfalfa (6.32), *Sorghum sudanense* (7.62) both pure grown and alfalfa grown under cover of *Sorghum bicolor* (7.96). The results obtained indicate that depending on the allelopathic soil activity of *Sorghum bicolor*, *Sorghum sudanense* and *Sorghum vulgare* var. *technicum,* they can be used for the growing of alfalfa under the cover as a measure for weed control in the year of establishment of the crop and for the increasing the productivity also. The equivalence between the allelopathic soil activity in pure grown crops, assessed by the degree of inhibition in the germination and initial development of *Lactuca sativa* L. as well as their use as cover crops for alfalfa were found.

Key words: Allelopathy, Cover crops, Rhizosphere soil, Sorghum species

In the last decades the research work was focused on the study of allelopathic interrelations between cultivated plants with the purpose of finding varieties with high allelopathic potential (Blum 2014).

In the ecology friendly farming system, the use of some grasses as cover crops (*Hordeum sativum, Avena sativa, Secale cereale*) in the year of establishment of alfalfa swards allows for reduction of the herbicides used. Cover crops have the weed suppressing ability and allow more efficient use of the area in the first year of cultivation of alfalfa (Chen *et al.* 2006). Sporadic reports include the use of crop cover or mixed alfalfa crops with species of the Sorghum genus, hough they form a high biomass yield in drought conditions and have significant allelopatic activity (Al-Suhaibani 2010, Iqbal *et al.* 2019). According to the researches of a number of authors part of the allelochemicals in the life cycle of the plants are formed by the root system and accumulated in the rhizosphere soil zone (Fujii *et al.* 2005). In many scientific studies it has been found that Sorghum species possess allelopathic potential due to the content of phenolic acids (Baerson *et al.* 2008a). There are also data on the presence of varietal differences in relation to the allelopathic potential of *Sorghum* species (Tibugari *et al*. 2018).

The purpose of this study was to assess the allelopaopathic activity of soil from the rhizosphere zone of alfalfa, grown under cover of *Sorghum bicolor* L. *Sorghum sudanense* Piper Staf. and *Sorghum vulgare* var. *technicum* Korn. on the initial development of *Lactuca sativa* L. and to detect the specimens with alleloopathic potential with a view to their inclusion as cover crops.

MATERIALS AND METHODS

For the field an experiment was used alfalfa (*Medicago sativa* L.) variety Dara created at the Institute of Forage Crops, Pleven, Bulgaria (Kertikov and Kertikova 2016). The variety has a high vigour, persistence and resistance to Fusarium and lucerne chalcid (Kertikova and Kertikov 2007, 2008, 2018). Two factors have been investigated: Factor A – alfalfa (*Medicago sativa* L.) grown under cover of Sorghum species: a_1– *Medicago sativa* L. variety Dara; a_2 – *Sorghum bicolor* L. variety Hercules; a_3– *Sorghum sudanense* Piper Staf., mutant form 300/43; a_4– *Sorghum vulgare* var. *technicum* Korn., local population from Bulgaria GL15A; a_5– *Medicago sativa* L. variety Dara + *Sorghum bicolor* L. variety Hercules; a_6 – *Medicago sativa* L. variety Dara + *Sorghum sudanense* Piper Staf., mutant form 300/43;

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a_7 – *Medicago sativa* L., variety Dara + *Sorghum vulgare* var. *technicum* Korn., local population from Bulgaria GL15A; a_8 – *Medicago sativa* L., variety Dara + *Sorghum vulgare* var. *technicum* Korn., local population from Bulgaria GL15A + *Sorghum sudanense* Piper Staf., mutant form 300/43. Factor B – concentrations: b_1 – Control untreated (0.0%); b_2–10.0%; b_3–20.0%; b_4–30.0% and b_5–50.0%. Soil sampling from the rhizosphere soil zone was done according to factor A in the BBCH 65-69 (Hess *et al*. 1997) following the methodology of Fujii *et al.* (2005) and Karmegam *et al.* (2014). To evaluate the allelopathic activity in the rhizosphere soil zone of the studied agrophytocenoses of alfalfa, grown under cover of *Sorghum* species in laboratory conditions the adapted method of Fujii *et al.* (2005) "Rhizosphere Soil Method" (RSM) was used.

In Petri dishes (90 mm), a soil is placed from the rhizosphere root zone of the studied agrophytocenoses according to Factor B. They are pipette 10 ml (0.8%) of the agar with added 1 ml/L $C_{10}H_{14}O$ as a chemical preservative (Marinov-Serafimov and Golubinova 2015). After its gelatinization, a second layer of 10 ml (0.8%) agar was pipette. For control, 0.8% agar is used with successive pipetting of two layers of 10 ml. The samples thus prepared are annealed for 72 h at $8^{\circ}C \pm 2^{\circ}C$. Then we put up 25 numbers of seeds from *Lactuca sativa* L. variety Great Lakes. The samples thus prepared were incubated at a temperature of $23^{\circ}C \pm 2^{\circ}C$ for 3 days. Each variant was five replicated. For assessing the results of the experiments following parameters were used.

Quantitative parameters: Number of germinated seeds in each treatment: percent of germination in each treatment (%).

Biometric parameters: Length of the root, stem and seedling, mm; fresh biomass in g per seedling. Length was measured using graph paper and the weight was recorded on an analytical balance.

Statistical evaluation and calculated formulas: germination seeds (GS%), the rate of emergence (GR%), the inhibition effects (IE) and the dynamic development index (DDI) were determined according to ISTA (1985) and response index (RI) according to Williamson and Richardson (1988). Growth rate and accumulation of fresh biomass of the root and germ was determined using an adapted formula by Dauta *et al.* (1990). The index of plant development (GI) was determined by Gariglio *et al.* (2002), seedling vigor index (SVI) by Islam *et al.* (2009), coefficient of allometry (CA) by Isfahan and Shariati (2007), respectively. The results obtained were calculated by programe product STATGRAPHICS Plus for Windows Version 2.1 at LSD P=0.05.

RESULTS AND DISCUSSION

The soil from the rhizosphere zone of alfalfa, grown under cover of Sorghum species has inhibition effect (1.3-33.0%) on the laboratory germination of the seeds of test-plant *Lactuca sativa* L. In terms of concentration dependencies, it is evident that by increasing the soil content from the rhizosphere zone (10.0-50.0% w/v) the percentage of germinated seeds of *Lactuca sativa* decreased (1.4-33.0%) as compared to the control variant and the differences were statistically significant at P=0.05, but only at higher concentrations (30.0 and 50.0% w/v) (Table 1).

The type and concentrations of the applied rhizosphere soil significantly affect the rate of emergency (GR%) of *Lactuca sativa.* A general tendency for decreasing the rate of emergence (GR%) of seeds of *Lactuca sativa* was found (4.3 to 59.7 times) for all variants compared to the lowest concentration studied (10% w/v). GR% ranged from 9.8-95.3% and depending on the type of the cover crop – the average lowest inhibition effects (42.5%) during the germination of the seeds was recorded in pure grown alfalfa, while the relatively highest (58.1%) in the case of alfalfa under cover of *Sorghum bicolor* (Table 2).

Depending on the type of donor (Factor A), the degree of inhibition on average for the variants studied in the germination of *Lactuca sativa* can be arranged in the following order: *Medicago sativa* (1.4%) → *Sorghum bicolor* (7.2%) → *Medicago sativa* + *Sorghum bicolor* (10.2%)

Table 1 Allelopatic effect of rhizosphere soil of alfalfa grown under cover of *Sorghum* species on germination and initial development of *Lactuca sativa* L. under laboratory conditions

Treatment	Germination (%)	Germination ($\%_C$)	Length, mm radix	hypocotyl	seedlings	Fresh biomass (g)
$Control_C$	67.2b		12.0b	13.2ab	25.2b	0.018
M. sativa	66.8	99.4	9.7	13.0	22.6	0.017
S. bicolor	65.2	97.0	8.1	10.9	19.0	0.015
S.sudanense	56.0	83.3	10.2	12.7	22.9	0.018
S. vulgare var. *technicum*	54.6	81.3	7.3	8.9	16.2	0.015
M. sativa + *S. bicolor*	60.4	89.8	11.2	12.2	23.4	0.015
M. sativa + *S. sudanense*	58.4	86.9	8.4	11.5	19.9	0.015
M. sativa + *S. vulgare* var. *technicum*	58.7	87.4	8.3	10.8	19.1	0.013
M. sativa + *S. bicolor* + *S. vulgare* var. *technicum*	52.7	78.5	6.8	10.6	17.4	0.014

Table 2 Index of development and coefficients of depression in germination and initial development of *Lactuca sativa* depending on the factors studied

Conc %	Germination				Length of seedlings					Fresh biomass, g for seedlings				*GI*
	DDI	*μ*	RI	GR%	DDI	*μ*	RI	SVI	CA	DDI	*μ*	RI	SVI	
M. sativa														
0.0	0.6	0.0	0.0	0.6	0.00	0.00	0.00	6.4	1.1	0.00	0.00	0.00	0. 3	100.0
10.0	19.2	0.0	0.0	19.2	-0.04	-0.01	-0.04	5.8	1.4	0.00	0.00	0.00	0. 3	95.6
20.0	1.5	0.0	0.0	1.5	-0.13	-0.03	-0.14	4.7	1.4	0.00	0.00	0.00	0.3	86.1
30.0	10.3	0.0	0.0	10.3	-0.10	-0.02	-0.10	5.1	1.3	0.00	0.00	0.00	0.3	89.7
50.0	0.0	0.0	0.0	0.0	-0.12	-0.03	-0.13	4.8	1.2	-0.05	-0.06	-0.01	0.3	85.9
S. bicolor														
0.0	3.0	-2.5	2.4	1.8	0.00	0.00	0.00	6.4	1.1	0.00	0.00	0.00	0.3	100.0
10.0	32.5	-19.8	16.5	25.8	-0.17	-0.04	-0.18	4.3	1.5	0.00	0.00	0.00	0.3	82.1
20.0	17.4	-19.3	16.2	9.5	-0.17	-0.04	-0.17	4.3	1.3	0.00	0.00	0.00	0.3	82.5
30.0	24.6	-18.9	15.9	17.5	-0.29	-0.07	-0.28	3.3	1.2	-0.29	-0.28	-0.07	0.2	69.1
50.0	16.7	-20.0	16.7	8.3	-0.38	-0.09	-0.35	2.7	1.4	-0.62	-0.50	-0.14	0.1	62.2
S. sudanense														
0.0	16.7	-19.3	16.2	8.6	0.00	0.00	0.00	6.4	1.1	0.00	0.00	0.00	0.3	100.0
10.0	15.0	4.9	-5.2	17.1	0.04	0.05	0.01	7.0	1.3	0.09	0.11	0.02	0.4	93.1
20.0	3.8	-2.4	2.3	2.7	-0.07	-0.08	-0.02	5.4	1.1	0.00	0.00	0.00	0.3	82.1
30.0	9.1	1.3	-1.3	9.7	-0.13	-0.13	-0.03	4.8	1.2	0.00	0.00	0.00	0.3	76.8
50.0	0.0	0.0	0.0	0.0	-0.21	-0.21	-0.05	4.0	1.4	-0.05	-0.06	-0.01	0.3	67.6
S. vulgare var. *technicum*														
0.0	18.8	-22.3	18.3	9.7	0.00	0.00	0.00	6.4	1.1	0.00	0.00	0.00	0.3	100.0
10.0	39.2	-32.9	24.7	29.2	-0.24	-0.05	-0.24	3.7	1.1	-0.10	-0.11	-0.02	0.3	70.3
20.0	32.6	-46.1	31.5	17.0	-0.32	-0.07	-0.30	3.1	1.1	-0.16	-0.17	-0.04	0.2	62.0
30.0	35.7	-39.5	28.3	23.0	-0.47	-0.11	-0.41	2.2	1.3	-0.22	-0.22	-0.05	0.2	48.8
50.0	16.7	-20.0	16.7	8.3	-0.59	-0.13	-0.48	1.7	1.5	-0.22	-0.22	-0.05	0.2	42.6
M. sativa +*S. bicolor*														
0.0	10.1	-10.6	9.6	5.4	0.00	0.00	0.00	6.4	1.1	0.00	0.00	0.00	0.3	100.0
10.0	6.7	13.4	-15.5	12.9	0.18	0.04	0.23	9.6	0.7	0.09	0.11	0.02	0.4	123.0
20.0	7.6	-6.6	6.2	4.5	-0.01	0.00	-0.02	6.2	1.2	0.00	0.00	0.00	0.3	94.3
30.0	7.1	3.4	-3.5	8.7	-0.19	-0.04	-0.19	4.1	1.5	-0.36	-0.33	-0.08	0.1	74.3
50.0	16.7	-20.0	16.7	8.3	-0.33	-0.07	-0.31	3.0	1.4	-0.62	-0.50	-0.14	0.1	57.0

→ *Medicago sativa* + *Sorghum vulgare* var. *technicum*, (12.6%) → *Medicago sativa* + *Sorghum sudanense* (13.1%) → *Sorghum sudanense* (16.6%) → *Sorghum vulgare* var. *technicum* (18.8%) → *Medicago sativa* + *Sorghum vulgare* var. *technicum* + *Sorghum sudanense* (18.8%) (Table 1).

These dependencies can be explained by the accumulation and excretion of allelochemicals saponins (glycosides) in the rhizosphere soil zone (Amali *et al.* 2014).

The observed differences in the inhibition effects in the germination of the seeds of *Lactuca sativa* can be explained by the diffusion of water-soluble alelchemicals and by the concentration of available soil samples from the rhizosphere zone in the medium (agar) (Sangeetha and Baskar 2015). In terms of concentration dependencies, it is evident that with an increase in the concentration of soil from the rhizosphere zone (20.0-50.0% w/v) the length of the root of *Lactuca sativa* decreases disproportionately in all variants compared to the control and the differences were statistically significant at P=0.05 (Table 2).

The soil from the rhizosphere zone of the investigated species, grown in mixed agrophytocenoses has a relatively weaker inhibition effect (0.9-10.2 times) on the growth of hypocotyl of test plant *Lactuca sativa* compared to the growth of the root (Table 1). The strongest depressing effect on the growth of hypocotyl in *Lactuca sativa* exerts a rhizosphere soil from the pure growing of *Sorghum vulgare* var. *technicum* Körn. Analogous were the results obtained in terms of increasing the length of the seedlings by the test-plant, following the established dependencies with respect to the dynamics of the growth of the root of *Lactuca sativa.* The mechanism of inhibition in the growth of seedlings of *Lactuca sativa* is due to the impact of the exudates of roots accumulated in the rhizosphere zone allelochemicals of the components in the studied agrophytocenoses, which according to Bais *et al.* (2006) is the result of the cell division and/or cell extension.

The accumulation of fresh biomass (g) for seedling in the initial stages of the development of *Lactuca sativa* depends on the same factors and follows the established dependencies in the increase in length of seedling by the difference that is less pronounced (Table 1). The statistical analyses showed that the integrational impact of the factors investigated exerts a retentive and/or inhibitory effect on the growth and accumulation of fresh biomass for seedling of test plant *Lactuca sativa*. The response index (RI) and the rate of growth and accumulation of fresh biomass of seedling (μ) depends on the type of donor (cover crop) and from the applied concentration of soil from the rhizosphere zone.

The relatively weakest allelopathic effect is found at the lowest concentration of 10.0% w/v and with its increase to 50.0% w/v, the dynamic development index (DDI), the response index (RI), the rate of increase and accumulation of fresh biomass (μ) and the coefficient of allometry (CA) of seedlings increase from 1.4-5.0 times, while seedling vigor index (SVI) decreases from 1.3-3.8 times compared to the control variant (Table 2).

The experimental data obtained confirm the findings of Baerson *et al.* (2008b) according to which the effect of allelochemicals is still evident in the germination of seeds, but it is more pronounced in the growth and accumulation of fresh biomass on the seedlings of test plants. A relatively weaker inhibitory effect IF_C was reported in the pure grown alfalfa and *Sorghum* species (IF_C 0.6-32.5%) compared to its growing under cover (IF_C 6.7-43.3). In the interaction donor (species of Sorghum genus) - (acceptor) *Medicago sativa* L. establishes a stimulating effect on the germination and initial development of *Lactuca sativa*. IE_D ranges from 2.4-46.1% with the exception of the growth of seedling and roots of test-plant in pure grown *Sorghum sudanense* and alfalfa under cover of *Sorghum sudanense,* where the inhibition effects were found (IE_D 1.3-13.4%). The reciprocal interaction *Medicago sativa* (acceptor) and donor (species of the Sorghum genus IE_A) established a inhibition effect in the test parameters IE_A from 2.3-31.5%, while in the pure grown *Sorghum sudanense* and alfalfa under cover of *Sorghum sudanense* statistically significant stimulative effect (IE_A 1.3-15.5%) was found.

The relatively highest common allelopathic effect $IE_{DA/C(average)}$ of soil from the rhizosphere was found in pure grown *Sorghum vulgare* var. *technicum* (17.44), as well as in the alfalfa grown under cover of *Sorghum vulgare* var. *technicum* (14.56) and *Sorghum bicolor* + *Sorghum vulgare* var. *technicum* (16.94), and the lowest was determined by the pure grown *Medicago sativa* (6.32) and *Sorghum sudanense* (7.62), as well as in alfalfa under cover of *Sorghum bicolor* (7.96). Intermediate positions occupy pure grown *Sorghum bicolor* (12.58) and *Medicago sativa* under cover of *Sorghum bicolor* (12.52). The soil from the rhizosphere zone of alfalfa, grown under cover of *Sorghum* species exerts inhibitory effect (1.3-33.0%) on the laboratory germination of the seeds of the test-plant *Lactuca sativa.*

The highest inhibition effect showed the soil from the rhizosphere zone of pure grown *Sorghum vulgare* var. *technicum* (17.44), alfalfa, grown under cover of *Sorghum vulgare* var. *technicum* (14.56) and under *Sorghum bicolor* + *Sorghum vulgare* var. *technicum* (16.94). The lowest inhibition effect showed the soil from the pure grown alfalfa (6.32), *Sorghum sudanense* (7.62) and alfalfa under cover of *Sorghum bicolor* (7.96). The results obtained indicate that depending on the allelopathic soil activity of *Sorghum bicolor*, *Sorghum sudanense* and *Sorghum vulgare* var. *technicum,* they can be used for the growing of alfalfa under the cover as a measure for weed control in the year of establishment of the crop and for the increasing the productivity also. The equivalence between the allelopathic soil activity in pure grown crops, assessed by the degree of inhibition in the germination and initial development of *Lactuca sativa* L. as well as their use as cover crops for alfalfa were found.

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