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ALLELOPATHIC ACTIVITY OF RHIZOSPHERE SOIL OF ALFALFA (*Medicago sativa* L.)

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Abstract

In the laboratory condition at the Institute of Forage Crops - Pleven was studied allelopathic potential of soil from the rhizosphere of alfalfa zone on germination and initial development of the test plants - Cucumis sativus L. It was found that concentrations of the rhizosphere soil zone on alfalfa have a stimulatory or inhibitory effect on the independent seedling growth of test plants. The rate of growth and accumulation of fresh biomass (μ), as well as the dynamic development index (DDI), response index (RI) and seedling vigor index (SVI) of seedlings depend mainly on applied concentration and do not depend on the type of the growth media. The observed differences in the values of the GI regarding the growth media - "distilled water" or "agar" can be explained by their different ability to dissolved and absorbed the available allelochemicals from rhizosphere soil samples of alfalfa as the comparisons between them are made under controlled conditions.

Key words*: alfalfa, allelopathic effect, rhizosphere soil, inhibition.*

INTRODUCTION

Allelopathic relationships in agrophytocenoses are determined by a variety of factors occurring simultaneously and/or sequentially, having direct or indirect effects on plant species through the synthesis of various chemical substances (allelochemicals) released into the environment (Rice, 1984; Seigler, 1996; Aleksieva & Serafimov, 2008; Ravlić et al., 2012; Kalinova et al., 2012; Ravlić et al., 2014; Baličević et al., 2015; Treber et al., 2015; Marinov-Serafimov, 2015a;2015b).

Alfalfa (*Medicago sativa* L.) is well known as a plant species with significant allelopathic potential, probably due to the accumulation and release of allelochemicals in the surrounding soil as a remedy against pests (Miller, 1983; Ells & McSay, 1991; Chon & Nelson, 2010; Mousavi et al., 2013). Many studies revealed that part of the allelochemicals (polyphenols, terpenoids, saponins, etc.), synthesized in the life cycle of alfalfa, are formed from the root system and accumulate into the rhizosphere zone (Ells & McSay, 1991; Wink & Mohamed, 2003; Wink, 2013). Other surveys have proved that the increment of allelochemicals concentration can influence their toxicity (Chung & Miller, 1995; Mousavi et al., 2013).

In the experimental work Wyman-Simpson (1991) found that alfalfa has a strong auto and hetero-toxic allelopathic effect, which is often used as a biological method for reducing the level of weed infestation in agricultural practice. It has been experimentally found that alfalfa root exudates strongly suppress the initial development of *H. vulgare; R. sativus; L. sativum; L. esculentum* and a number of monocotyledonous and dicotyledonous weed species (Rice, 1984; Ming Chung & Miller, 1995; El-Dariera & El-Dienb, 2011).

Oleszek & Jurzysta (1987) reported that high amounts of glycosides of the medicagenic acid accumulate in the alfalfa roots, accounting for about 6% of the dry underground biomass. Assuming that, they calculated that the alfalfa crops can accumulate up to 10 t per ha of root biomass in the soil, which is equivalent to introducing about 600 kg per ha of highly

active allelopathic compounds, which could affect the cultivation of subsequent crops.
The aim of the present study was to determine the allelopathic activity of soil from the alfalfa rhizosphere zone on the initial development of test plant species *Cucumis sativus* L.

MATERIALS AND METHODS

The study was conducted at laboratory conditions in the Institute of Forage Crops, Pleven, Bulgaria. Two factors were studied: Factor A - growth media: a1 - Distilled water; a2 - Agar-agar, and Factor B - concentration: b1 - Control; b2 - 2.5% w/v; b3 - 5.0% w/v; b4 - 10.0% w/v and b5 - 5.0% w/v.
Sampling of soil samples from the rhizosphere zone was carried out in a five-year alfalfa stand (Dara variety) in phenophase BBCH 65-6.
Each soil sample, consisted of 5 subsamples, was air-dried and stored in polyethylene containers at 4°C. Two screening methods were used to detect the presence of allelochemicals in the alfalfa rhizosphere soil: 1) Cold Aqueous Extracts Method; 2) Rhizosphere Soil Method (RSM).
The experimental method of cold aqueous extract was applied as follows: 20 g of the alfalfa rhizosphere soil were soaked in 100 ml of distilled water. These samples were cold extracted at 22 ± 2°C for 24 h. The extracts obtained were decanted, filtered through Filtrak 389 filter paper and brought to final concentrations of 2.5, 5.0, 15.0 and 20.0% w/v. $C_{10}H_{14}O$ was added to each of the extracts (Marinov-Serafimov et al., 2007). Ten seeds of *Cucumis sativus* L. (Longo da China variety) were placed into petri dishes (90 mm diameter) between Filtrak 389 filter paper disks. The seed surface was pre-sterilized for one minute with a 0.3% $KMnO_4$ solution and then dried. The experimental extracts have been added at the quantity of 20 ml per petri. Distilled water was used for the control. The petri dishes were placed in a thermostat at 22°C ± 2°C for five days. Each variant is set in four replications.
The adapted method of RSM, proposed by Fujii et al. (2005) and Karmegam et al. (2014), was applied in order to determine the presence of allelochemicals, separated from the root system in the alfalfa rhizosphere soil zone: 2.5, 5.0, 10.0 and 20.0% w/v of the alfalfa rhizosphere soil (equivalent to 3.3, 6.7, 20.0 and 26.7 cm^3 volume) were placed into Petri dishes (90 mm diameter). Then 10 ml agar (1%) with 1 ml/L^{-1} $C_{10}H_{14}O$ have been added into each Petri (Marinov-Serafimov et al., 2007). After gelation, a second layer of 10 ml agar (1%) was pipetted. 1% agar with sequential pipetting of two 10 ml layers was used for the control. All petri dishes were placed into a heat shaker for 24 h at 22°C ± 2°C. After that, 10 seed of *Cucumis sativus* L. (Longo da China variety) were added and all petri dishes were placed in a thermostat at 22°C ± 2°C for five days. Each variant is set in four replications.
The following parameters were determined for all variants of the experiment:

- percentage of germinated seeds (%);
- seedling length (root + shoot), cm;
- fresh biomass of seedling, g;
- Dynamic Development Index (DDI): $DDI = \left\{\frac{t\log^2}{\log b - \log a}\right\}$, where: a is the percentage of germinated seeds (%), length (cm) and/or biomass (g) of seedlings in the control variant; b - seed germination (%), length (cm) and/or biomass (g) of seedlings in the experimental variant; t - duration, days'.
- Allelopathic Effect Index (RI), according to the formula of Williamson & Richardson (1988): $RI = \frac{T}{C} - 1$, where: C - parameter in the control variant; T - parameter in the experimental variant;
- Rate of growth and accumulation of fresh seedlings biomass was determined by the adapted formula of Dauta et al. (1990): $\mu = \left\{\frac{\ln N_t - \ln N_0}{t}\right\}$, where: *Nt* - length (cm) or biomass (g) of seedlings in the experimental variant; *No* - length (cm) or biomass (g) of seedlings in the control variant; t - duration, days;
- Growth Rate (GR) was calculated as follows - $GR_{\%} = \left(1 - \frac{(N_{t-C_n})}{(N_c)}\right).100$, where: *Nt* - percentage of the germinated seeds in the experimental variant, %; *Nc* - germinated seeds in the control, %; *Cn* - concentration, %;
- Development Index (GI) according to Gariglio et al. (2002): $GI = \left[\left(\frac{G}{G_0}\right).\left(\frac{L}{L_0}\right)\right].100$, where: *G* - germinated seeds in experimental variant, %; G_0 - germinated seeds in the control variant, %; *L* - length of seedlings in

experimental variant, converted to percentage; L_0 - length of seedlings in the control variant, accepted for 100%;

- Seedling vigour index (SVI) according to Islam et al. (2009) : $SVI = \left(\frac{S.G}{100}\right)$, where: S - length (cm) or biomass (g) per seedling; G - germinated seeds,%;
- Coefficient of Allometry (CA) according to Nasr & Shariati (2007): $CA = \frac{L_S}{L_r}$, where: L_s - length (cm) or biomass (g) per shoot; L_r - length (cm) or biomass (g) per root.

The percentage of germinated seeds was transformed according to the formula of Hinkelmann & Kempthorne (1994): $Y = \arcsin\sqrt{x_{\%}/100}$.

The results obtained were processed mathematically and statistically with the software products STATGRAPHICS Plus for Windows Version 2.1 and Statistica 10.

RESULTS AND DISCUSSIONS

A general tendency to decrease the percentage of germinated seeds of the test plants with increasing the concentration (Factor B) in the growth media was observed (Table 1). The degree of inhibition of seed germination, depending on the type of growth media (Factor A) - distilled water or agar, varied from 0 to 23.6% in the distilled water variants (a1) and from 0 to 30.5% in the agar variants (a2). No significant differences in the seed germination rate (GR%) according to the growth media type have been found, whereas the dynamic development index (DDI), the allelopathic effect index (RI) and the growth rate (μ) differed significantly (from 0.2 to 4.3 times) (Table 2). These differences probably could be explained by the different solubility of allelochemicals in the growth media.

Data from the biometric measurements on root, shoot and seedling length (cm) make it possible to objectively evaluate the allelopathic potential of alfalfa rhizosphere soil (Tables 1 and 2), depending on the concentrations applied and the type of growth media. Two highest concentrations of 10 and 20% w/v of alfalfa rhizosphere soil have a significant inhibitory effect ranging from 3.7 up to 89.9% (P = 0.05), regardless of the type of media. By reducing the concentration to 2.5 and 5.0% w/v, the inhibitory effect on the root, shoot and seedling (cm) also was reduced and practically exerted an indifferent and/or weak stimulating effect from 1.0 to 20.2% on both growth media when compared to the control variants. This dependence could be explained by the presence of secondary metabolites - glycosides, tannins, polyphenols, terpenoids, saponins, and other allelopathically active substances, synthesized in the life cycle of alfalfa and separated from the root system in the rhizosphere zone.

Table 1. Allelopathic effect of rhizosphere soil of alfalfa on germination and initial development of *Cucumis sativus* L.

Variants			Parameters					
Growth medium/ Concentration, % w/v		Germination,% ± SE	Length, cm			Biomass, g		
			root ± SE	shoot ± SE	seedling ± SE	root ± SE	shoot ± SE	seedling ± SE
Distilled water	0.0	83.4a ±6.65	1.04 bc ±0.02	0.83b ±0.07	1.87b ±0.09	0.08a ±0.006	0.11b ±0.010	0.19c ±0.014
	2.5	83.4 a ±6.65	1.20 cd ±0.05	0.74b ±0.1	1.93b ±0.14	0.04b ±0.009	0.10b ±0.008	0.14b ±0.009
	5.0	76.7 a ±6.68	1.32 d ±0.08	0.78b ±0.08	2.10b ±0.16	0.04b ±0.007	0.10b ±0.010	0.14b ±0.016
	10.0	76.7 a ±6.68	0.87 ab ±0.15	0.30a ±0.02	1.17a ±0.03	0.03b ±0.004	0.06a ±0.009	0.08a ±0.010
	20.0	63.7 a ±9.24	0.78 a ±0.12	0.26a ±0.05	1.04a ±0.17	0.03b ±0.004	0.05a ±0.011	0.08a ±0.010
Agar-agar	0.0	83.4a ±3.31	1.14b ±0.03	0.99b ±0.06	2.13b ±0.09	0.09b ±0.013	0.15b ±0.029	0.24b ±0.032
	2.5	83.4a ±3.31	0.91b ±0.07	1.00b ±0.18	1.91b ±0.21	0.08b ±0.007	0.15b ±0.021	0.23b ±0.025
	5.0	58.0a ±4.22	1.29b ±0.63	0.92b ±0.10	2.21b ±1.03	0.03a ±0.02	0.06a ±0.058	0.09a ±0.077
	10.0	58.0a ±4.22	0.38a ±0.05	0.27a ±0.11	0.65a ±0.13	0.02a ±0.0061	0.04a ±0.029	0.06a ±0.028
	20.0	58.0a ±4.22	0.18a ±0.15	0.10a ±0.20	0.28a ±0.35	0.02a ±0.019	0.01a ±0.025	0.03a ±0.044

Legend: a, b, c, d LSD at P = 0.05 level, ± SE standard error

Table 2. Indexes of germination and initial development of the test plant depending on the two studied factors

Variant		Germination				Seedling length					Seedling biomass					
Growth medium/ Concentration,% w/v		*DDI*	*RI.10²*	*μ.10²*	*GR%*	*DDI*	*RI.10²*	*μ.10²*	*SVI.10² cm*	*CA cm*	*DDI*	*RI.10²*	*μ.10²*	*SVI.10⁴ g*	*CA g*	**GI**
Distilled water	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	3.5	79.8	0.0	0.0	0.0	3.61	1.38	100.0
	2.5	0.0	0.0	0.0	97.0	35.6	-9.4	3.2	3.7	61.7	-3.7	-26.3	-6.1	1.96	2.50	103.2
	5.0	-13.4	-8.0	-1.7	86.0	9.7	-1.4	12.3	4.4	59.1	-3.7	-26.3	-6.1	1.96	2.50	103.3
	10.0	-13.4	-8.0	-1.7	80.0	-2.4	-45.1	-37.4	1.4	34.5	-1.3	-57.9	-17.3	0.64	2.00	57.5
	20.0	-4.2	-11.8	-5.4	52.4	-1.9	-51.2	-44.0	1.1	33.3	-1.3	-57.9	-17.3	0.64	1.67	42.5
Agar-agar	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	4.5	0.87	0.0	0.0	0.0	5.76	1.67	100.0
	2.5	0.0	0.0	0.0	97.0	-10.3	-10.3	-2.2	3.6	1.10	-26.4	-4.2	-0.9	5.29	1.88	89.7
	5.0	-3.1	-30.5	-7.3	63.5	30.5	3.8	0.7	4.9	0.71	-1.1	-62.5	-19.6	0.81	2.00	72.2
	10.0	-3.1	-30.5	-7.3	57.6	-0.9	-69.5	-23.7	0.4	0.71	-0.8	-75.0	-27.7	0.36	2.00	21.2
	20.0	-3.1	-30.0	-7.3	45.6	-0.6	-87.0	-40.6	0.1	0.56	-0.5	-88.0	-41.6	0.09	0.50	9.1

It is well known that alkaloids, tannins, and some other secondary metabolites exhibit a toxicity with protoplasmic and haemolytic activity (Li, 2010; Zohaib et al., 2014). At higher concentrations (10.0 and 20.0% w/v) they caused an inhibitory effect on the test plants development, while at lower concentrations (2.5 and 5.0% w/v) they exerted an indifferent and/or stimulating effect to different extent.

The dynamics in the accumulation of fresh biomass in g per seedling through the initial stages of *C. sativus* development depended on the same factors and followed the observed dependences on root, shoot and seedling growth (cm) (Table 1).

The highest amount of fresh biomass in g per seedling was accumulated at the lower concentrations of 2.5 and 5.0% w/v. A tendency to weight decrease was found with concentration increment to 10.0 and 20.0% w/v as follows: in the distilled water media the biomass inhibition ranged from 1.1 up to 2.3 times, while in the agar media it ranged from 1.1 to 8.0 times (P = 0.05). An exception of this tendency was found at the lowest concentration of 2.5% w/v in agar variant, as well as at the two lower concentrations of 2.5 and 5.0% w/v in the distilled water variant.

Statistical evaluation of the results showed that the applied concentrations of alfalfa rhizospheric soil have a stimulating, inhibitory or indifferent effect on the seedling growth. The growth rate and accumulation of fresh biomass (μ), development index (DDI), allelopathic effect index (RI) and seedling vigour index (SVI) in test seedlings depended mainly on the concentration applied and are independent of the type of growth media (Table 2). Similar results have been obtained in relation to the coefficient of allometry (CA) with respect to the seedling length - increasing of concentration led to the CA decrease from 1.3 to 2.4 times, regardless of the type of media. When regarding the CA with respect to the biomass formation, its value increased from 0.5 to 3.3 times compared to the control variant. The analysis of the results obtained showed that the coefficients of depression according to Factor A (growth media) and Factor B (concentration) are correlated only at higher concentrations (r varies from –0.757 to –0.992).

The complex assessment of tested rhizosphere soil extracts showed that depending on the GI values, they could be arranged in the following order: distilled water media - from 103.2 to 42.5%, and agar media - from 89.7 to 9.1%. Therefore, the observed differences could be explained by their different ability to dissolve and absorb the available allelochemicals from soil samples, collected in the alfalfa rhizosphere zone, since comparisons between them were made at controlled conditions.

Data from the dispersion analysis express the hierarchical distribution of variation to determine the weight of factors η^2 on the laboratory germination. They indicate that Factor A (growth media) and Factor B (concentration) have a relatively equal proportion of the total variation, and η^2 is in the range of 5.05 to 5.78 (Figure 1). The interaction of the studied A x B factors accounts for a relatively small proportion of the total variation $\eta^2 = 1.23$, with no significant differences.

With regard to the hierarchical distribution of variation between the factors determining growth (cm) and the accumulation of fresh

biomass in g per seedling, the weight of factor B accounts for the largest proportion of the total variation and the weight of the factor (η^2) is in the range of 55.9 to 75.32. The influence of the media (Factor B) occupies a relatively small share of the total variation, with a factor weight (η^2) from 0.03 to 9.95. The variance due to the A x B relationships have a relatively high proportion of the total variation, and the η^2range is from 16.93 to 23.76 (Figure 1).

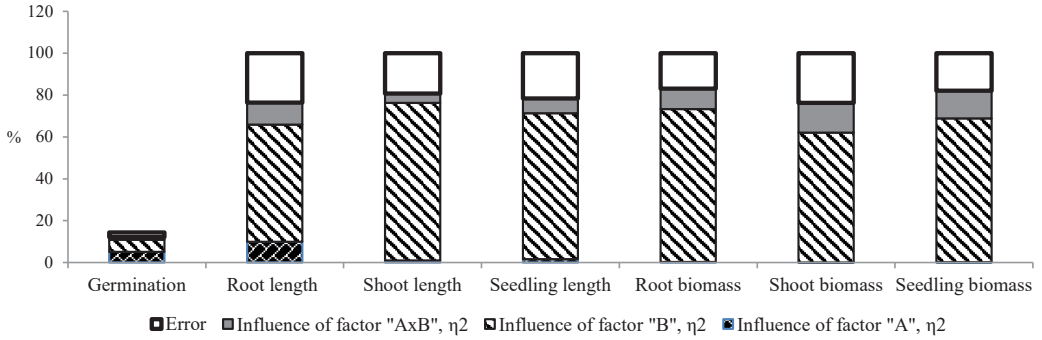

Figure 1. Dispersion analysis of the two factors

CONCLUSIONS

Soil from the rhizosphere zone of alfalfa (Dara variety) has a slight inhibitory effect on seed germination of the test plants, depending on the type of growth media. The applied concentrations of the alfalfa rhizosphere soil have a stimulating, inhibitory or indifferent effect on the seedlings growth. The rate of growth and accumulation of fresh biomass (μ), the development index (DDI), allelopathic effect (RI) and seedling vigour index (SVI) depend mainly on the concentration applied and are independent of the type of media.

The complex assessment of tested rhizosphere soil extracts showed that the allelopathic inhibition was stronger in the distilled water media - from 103.2 to 42.5%, while in the agar media it ranged from 89.7 to 9.1%. Therefore, the observed differences could be explained by their different ability to dissolve and absorb the available allelochemicals from soil samples, collected in the alfalfa rhizosphere zone, since comparisons between them were made at controlled conditions.

REFERENCES

Aleksieva, A., Serafimov, P. (2008). A study of allelopathyc effect of *Amaranthus retroflexus* (L.) and *Solanum nigrum* (L.) in different soybean genotypes. *Herbologia, 9*(2), 47–58.

Baličević, R., Ravlić, M., Mišić, M., & Mikić, I. (2015). Allelopathic effect of *Aristolochia clematitis* L. *Proceedings of the 50th Croatian and 10th International Symposium on Agriculture*, 54–58.

Chon, S., Nelson, C. (2010). Allelopathy in Compositae plants. A review. *Agronomy for Sustainable Development, 30*(2), 349–358.

Chung, I., Miller, D. (1995). Effect of alfalfa plant and soil extracts on generation and seedling growth. *Agronomy Journal, 87*, 762–767.

Dauta, A., Devraux, J., Piquemal, F., Boumnich, L. (1990). Growth rate of four freshwater algae in relation to light and temperature. *Hydrobiologia, 207,* 221–226.

El-Dariera, S., El-Dienb, M. (2011). Biological activity of *Medicago sativa* L. (alfalfa) residues on germination efficiency, growth and nutrient uptake of *Lycopersicon esculentum* L. (tomato) seedlings. *Journal of Taibah University for Science, 5*, 7–13.

Ells, J., McSay, A. (1991). Allelopathic Effects of Alfalfa Plant Residues on Emergence and Growth of Cucumber Seedlings. *Horticural Science, 26*(4), 368–370.

Fujii, Y., Furubayashi, A., Hiradate, S. (2005). Rhizosphere soil method: a new bioassay to evaluate allelopathy in the field. *Proceedings of the 4th World Congress on Allelopathy "Establishing the Scientific Base", Wagga Wagga*, 490–492.

Islam, A., Anuar, N., Yaakob, Z. (2009). Effect of genotypes and pre-sowing treatments on seed germination behavior of Jatropha. *Asian Journal of Plant Science, 8*, 433–439.

Gariglio, N, Buyatti, M., Pillati, R, Rossa, D., Acosta, M. (2002). Use a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. *New Zealand Journal of Crop and Horticulture Science, 30*, 135–139.

Hinkelmann, K., Kempthorne, O. (1994). *Design and analysis of experiments*. New York: Wiley and Sons.

Kalinova, S., Golubinova, I., Hristoskov, A., Ilieva, A. (2012). Allelopathic effect of aqueous extract from root systems of Jonson grass on seed germination and the initial development of soybean, pea and vetch. *Ratarstvo i povrtarstvo, 49*(3), 250–256.

Karmegam, N., Kalpana, M., Prakash, M. (2014). Allelopathic effect of aqueous root bark extract of *Tamarindus indica* L. and rhizosphere soil on germination and seedling growth of *Oryza sativa* L. *International Journal of Current Microbiology and Applied Sciences, 3*(3), 505–514.

Li, Z.H., Wang, Q., Ruan, X., Pan, C.D., & Jiang, D.A. (2010). Phenolics and Plant Allelopathy. *Molecules, 15*, 8933-8952.

Marinov-Serafimov, P., Dimitrova, T., Golubinova, I., Ilieva, A. (2007). Study of suitability of some solutions in allelopathic researches. *Herbologia, 8*(1), 1–10.

Marinov-Serafimov, P., Golubinova, I., Katova, A. (2015a). Allelopathic activity of rhizosphere soil at some annual cereal forage crops. *Proceedings of The Union of Scientists - Ruse, Section "Agriculture & Veterinary Sciences", 3*(7), 202–208.

Marinov-Serafimov, P., Katova, A., Golubinova, I. (2015b). Allelopathic activity of rhizosphere soil in some perennial grasses. *Proceedings of The Union of Scientists - Ruse, Section "Agriculture & Veterinary Sciences", 3*(7), 209–215.

Miller, D.A. (1983). Allelopathic effects of alfalfa. *Journal of Chemistry and Ecology, 9*(8), 1059-1072.

Ming Chung, I., Miller, D. (1995). Natural Herbicide Potential of Alfalfa Residue on Selected Weed Species. *Agronomy Journal, 87*(5), 920–925.

Mousavi, S., Alami-Saeid, K.H., Moshatati, A. (2013). Effect of leaf, stem and root extract of alfalfa (*Melilotus indicus*) on seed germination and seedling growth of wheat (*Triticum aestivum*). *International Journal of Agriculture and Crop Sciences, 5*(1), 44–49.

Nasr, M., Mansour, S. (2005). The use of allelochemicals to delay germination of *Astragalus cycluphyllus* seeds. *Journal of Agronomy, 4*(2), 147–150.

Oleszek, W., Jurzysta, M. (1987). The allelopathic potential of alfalfa root medicagenic acid glycosides and their fate in soil environments. *Plant and Soil, 98*(1), 67–80.

Ravlić, M., Baličević, R., Knežević, M., Ravlić, I. (2012). Allelopathic effect of scentless mayweed and field poppy on seed germination of winter wheat and winter barley. *Herbologia, 13*(2), 1–7.

Ravlić, M., Baličević, R., Lucić, I. (2014). Allelopathic effect of parsley (*Petroselinum crispum* Mill.) cogermination, water extracts and residues on hoary cress (*Lepidium draba* (L.) Desv.). *Poljoprivreda, 20*(1), 22–26.

Rice, E.L. (1984). *Allelopathy.* 2nd Edition. Orlando: Academic Press.

Seigler, D. (1996). Chemistry and Mechanisms of Allelopathic Interactions. *Agronomy Journal, 88*, 876–885.

Treber, I., Baličević, R., Ravlić, M. (2015). Assessment of allelopathic effect of pale persicaria on two soybean cultivars. *Herbologia, 15*(1), 31–38.

Wink, M., Mohamed, G.I.A. (2003). Evolution of Chemical Defense Traits in the Leguminosae: Mapping of Distribution Patterns of Secondary Metabolites on a Molecular Phylogeny Inferred from Nucleotide Sequences of the rbcL Gene. *Biochemical Systematics and Ecology, 31*, 897–917.

Wink, M. (2013). Evolution of Secondary Metabolites in Legumes (Fabaceae). *South African Journal of Botany, 89*, 164–175.

Williamson, G., Richardson, D. (1988). Bioassays for allelopathy: measuring treatment responses with independent controls. *Journal of Chemistry and Ecology, 14*, 181–188.

Wyman-Simpson, C., Waller, G., Jurzysta, M., McPherson, J., Young, C. (1991). Biological activity and chemical isolation of root saponins of six cultivars of alfalfa (*Medicago sativa* L.). *Plant and Soil, 135*(1), 83–94.

Zohaib, A., Tanveer, A., Khaliq, A., Safdar, M. (2014). Phytotoxic effect of water soluble phenolics from five Leguminous weeds on germination and seedling growth of Rice. *Pakistan Journal of Weed Scientific Research, 20*(4), 417–429.