

Allelopathic tolerance of pea cultivars to *Sorghum halepense* L. (Pers.) extracts

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SUMMARY

In order to evaluate the allelopathic effect of *Sorghum halepense* extracts on germination and initial growth of six pea (*Pisum sativum* subsp. *sativum*, *Pisum sativum* subsp. *arvense*) cultivars and to identify tolerant cultivars, a laboratory experiment was conducted. The studied cultivars revealed different levels of susceptibility to allelopathic impact of root and aboveground biomass extracts of *S. halepense*. Root growth parameters (length and weight) of the pea cultivars exhibited greater susceptibility to weed extracts than stem parameters. The inhibitory effects of the extracts on germ length of *P. sativum* ranged from 1.4% (cultivar Mir) to 45.0% (Kamerton), on germ weight – from 3.5% (Pleven 4) to 42.9% (K-80), and on seed germination – from 11.8% (Mir) to 31.3% (K-80). Total inhibitory effect, i.e. the impact of *S. halepense* extracts on all studied parameters of *P. sativum*, revealed that the cultivars Mir and Pleven 4 were the most tolerant. Growing such cultivars may reduce weed damage. Low tolerance was manifested by the cultivar K-80, while Modus, Glyans and Kamerton ranked intermediate. The cultivars with large-size seeds or lower grain protein content were more affected by the depressing effect of *S. halepense* extracts.

Keywords: Allelopathy; *Sorghum halepense*; Pea; Germination; Growth

INTRODUCTION

High weed infestation of agricultural fields with perennial and annual weeds leads to lower crop yields. Allelopathic relationships between crops and weeds are greatly responsible for yield losses. Weeds influence crop growth by causing phytotoxicity from fallen seeds, leaves, flowers, decomposition of plant residues, exudates, air and water discharges, etc. Harmful impacts of weeds can be assessed via three groups of factors – the type of weed, extent and duration of weed infestation, and biological characteristics of relevant crops (Stoimenova et al., 2008).

Sorghum halepense L. (Pers.) is considered an economically important and most widespread weed in areas where major agricultural crops are grown in Bulgaria (Tonev et al., 2008; Kalinova et al., 2012; Hristoskov, 2013). It belongs to a group of weeds with evidenced allelopathic effects. The inhibitory effect of plant extracts of various johnsongrass parts results from the presence of chlorogenic acid, *p-coumaric acid*, *oxybenzaldehyde*, senile acid (Rice, 1995; Sari et al., 1999), phenols and tannins (Lyubenov, 1984). Phenolic acids cause destruction of mineral ions, depolarization of the plasmalemma, and increased membrane permeability violations in all of plant metabolism (Einhellig, 1986).

Allelopathy also has a potential to be used in breeding programmes for biological control against weeds through the development of less susceptible genotypes or ones with high allelopathic potential (Ebana et al., 2001). Differences among genotypes and cultivars have been found in many crops, both those sensitive to various weed species, e.g., soybean and maize (Baličević et al., 2014; Treber et al., 2015), and crops with allelopathic potential against weeds, e.g. sorghum and sunflower (Alsaadawi & Dayan, 2009; Alsaadawi et al., 2012), as their susceptibility depends both on the weed species and the cultivar (Verma & Rao, 2006). Genotypic variation in allelopathic tolerance also has been detected in wheat, rice and some other crops (Rice, 1995; Bashir et al., 2012).

The present study was conducted to evaluate the allelopathic effect of different *S. halepense* extracts on germination and initial growth of pea cultivars, and to identify the cultivars tolerant to chemicals produced by the weed.

MATERIALS AND METHODS

A laboratory experiment was conducted at the Institute of Forage Crops (Pleven, Bulgaria) in 2015.

Seed and plant material

Six pea cultivars were examined in the study (factor A): Glyans, Kamerton, Modus, Pleven 4 (belonging to *Pisum sativum subsp. sativum*), Mir and K-80 (belonging to *Pisum sativum subsp. arvense*). The seeds of the studied cultivars were harvested in 2014. The main characteristics of seeds are presented in Table 1.

Table 1. Crude protein content and 1000 seeds weight of the studied pea cultivars

Cultivar	Crude protein, %	1000 seeds weight, g
Kamerton	28.42	200.56
Glyans	24.25	224.82
Pleven 4	28.61	151.00
K 80	26.33	182.40
Mir	27.83	107.70
Modus	26.18	208.00

The aboveground and root biomass of *S. halepense* was collected from naturally infested fields at the phenological stage BBCH 65 of the weed (Hess et al.,

1997). Fresh material was cut into 1 cm pieces, oven dried at 60 °C to constant dry weight and ground into fine powder.

Weed extracts preparation

Water extracts were prepared according to Norsworthy (2003) by soaking 100 g of plant powder in 1000 ml of distilled water, and then keeping the mixtures at 24 ± 2 °C for 24 h. The mixtures were filtered through muslin cloth and after that through filter paper. The obtained extracts were diluted with distilled water to the final concentrations of 1.25, 2.5, 5.0 and 10.0%.

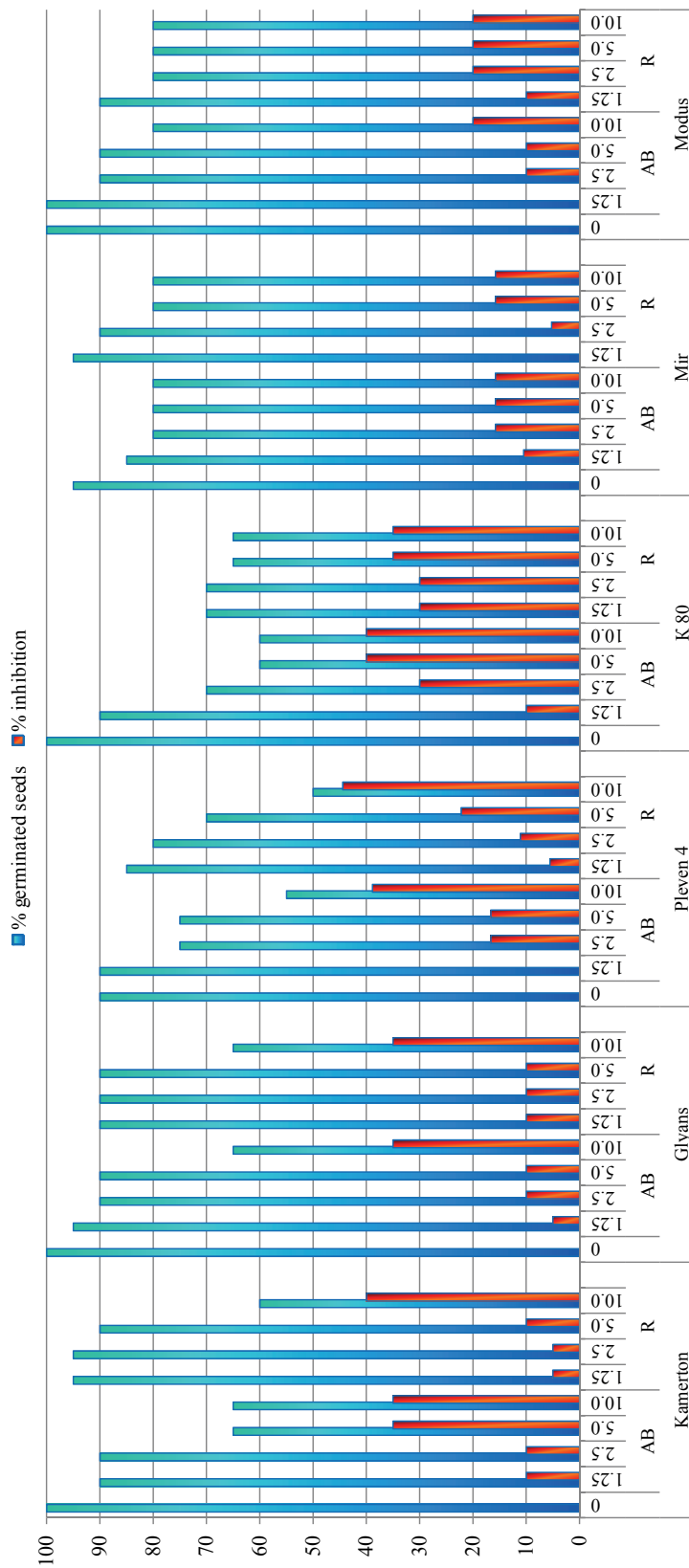
Bioassay techniques and estimation

Twenty seeds of each pea cultivar were placed on top of filter paper in each Petri dish (9 cm in diameter). The extracts were pipetted at 1:6 ratio to seed weight in Petri dishes (Marinov-Serafimov et al., 2007), while distilled water was used as a control. All treatments had four replications. Petri dishes were kept in a thermostat at 22 ± 2 °C temperature for seven days. The following indicators were measured: germination (%), seedling length (root and stem) (cm), fresh weight of root, stem and germ (g).

The obtained data were statistically processed using the software Statgraphics Plus for Windows Ver. 2.1 at LSD 0.05%.

RESULTS AND DISCUSSION

Germination is one of the most important plant growth stages and severely affected by allelochemical components (Bogatek et al., 2006). The water extracts of aboveground and root biomass of *S. halepense* showed inhibitory effects on seed germination of the six studied cultivars (Figure 1). The percentage of germinated seeds in different combinations varied from 50 to 100%, and the highest average values were observed in the cultivars Modus, Glyans and Mir (86.3, 84.4 and 83.3%, respectively). High susceptibility to the weed extracts was exhibited by cvs. K-80 and Pleven 4, in which this indicator had average values of 68.8 and 72.5%, respectively. All tested concentrations had reducing effects on germination, except the seed treatment of cv. Modus with 1.25% extract of aboveground biomass, in which no differences were observed compared to the control. In confirmation of a number of previous studies, the increasing extract concentrations enhanced their suppressive effect (Yang et al., 2007; Georgieva et al., 2008; Chen et al., 2011; Kuang et al., 2014).



AB – aboveground biomass; R – roots

Figure 1. Influence of *Sorghum halepense* extracts on seed germination of *Pisum sativum* cultivars

Under the laboratory conditions of this experiment, the concentrations of 1.25, 2.5, 5.0 and 10.0% of *S. halepense* inhibited *P. sativum* germination, respectively: 8.0, 14.5, 20.0 and 31.2%, while no significant differences were detected regarding the type of extract. The highest tolerance to the activity of weed extracts at germination was shown by seeds of the cultivars Mir and Modus, in which the percentage of inhibition had the lowest values (11.8 and 13.8%, respectively).

The suppressive effects of weed extracts on germ (stem + root) growth of *P. sativum* was stronger than on seed germination as the former average for all cultivars was 34.0% and ranging from 0.2 to 75.2% (Table 2). The lowest concentration (1.25%) of aboveground biomass had a weak stimulating effect on germ length of cvs. Glyans and Modus. In cv. Mir, the lowest concentration (1.25%) of aboveground and root biomass had a significantly high stimulating activity – 33.4 and 26.5%, respectively, over the control value. The same cultivar manifested a very high tolerance to the activity of the tested extracts since the reduction in germ length, compared to the control, was only 1.4%. Pleven 4 and Modus showed an average tolerance (reduction of 31.7 and 37.7%, respectively), and cvs. K-80, Glyans and Kamerton low tolerance (41.7, 46.3 and 45.0%, respectively).

Generally, the differences in germ length in all treated variants, compared to the control, were significant with the following exceptions: 1.25% aboveground biomass extract in cvs. Glyans and Modus; 2.5% and 5.0% extracts from aboveground and root biomass in cv. Mir.

Comparing the effects of extracts of different parts of *S. halepense* on the germ length of all cultivars, a significantly stronger allelopathic effect of the root extract of the weed was detected, compared to the aboveground biomass, as the reducing effects were 42.8 and 25.1 %, respectively. According to Iman et al. (2006), the strength of allelopathic activity of a particular plant part probably depends on the presence of various allelopathic compounds (or their high concentrations). The mechanism itself of growth inhibition resulting from the action of allelochemicals is based on a reduction in cell division (Iman et al., 2006) and cell membrane disorders (Rice, 1984).

Considering the influence of aqueous extracts of *S. halepense* on root and stem growth of different pea cultivars, root growth was negatively affected more than stem growth. The reducing effect on root length was 3 times higher, and ranged from 30.1 to 64.3% on average in different pea cultivars, while the corresponding stem growth reduction was from 9.8 to

22.9%. The cultivar Mir demonstrated tolerance in terms of both growth indicators. In all cultivars, 1.25% extracts of aboveground biomass of *S. halepense* had a stimulating effect on stem growth, and in some cultivars (Modus and K-80) a similar effect was caused by root extracts of the weed. According to Ahmed et al. (2001), stimulatory effects of low concentrations suggest that weed infestation with this weed in low thresholds will not influence considerably the seed germination and plant development.

The results showing a higher inhibitory effect of weed extracts on pea root length than stem length are consistent with the results from studies of a number of other authors, who revealed the same relationship in other weed species – *Amaranthus* spp. (Qasem, 1995), *Polygonum lapathifolium* (Baličević et al., 2013), *Datura stramonium* (Elisante et al., 2013), *Chenopodium album* and *Raphanus sativus* (Aryakia et al., 2015). According to some researchers (Iman et al., 2006), high susceptibility of the root of crop species to the allelopathic effect of weeds is due to its direct contact with the extracts during experiments. Suppressed root growth probably affects the physiological and biological functions of the plant as a mechanical stabilization in the soil, and absorption of water and other essential nutrients necessary for its growth and development.

According to An et al. (1998), this could lead to a decrease in fresh and dry weight of the test species. The results in this experiment also showed that the six studied pea cultivars sustained a significant reduction in fresh seedling weight after treatment with *S. halepense* extracts, varying from 0.9 to 69.9%. An exception, as for previous indicators, was observed only at the lowest concentration of extracts, which exhibited a stimulating effect in some of the cultivars (Glyans, Pleven 4, Mir, Modus). The rising concentrations of 2.5, 5.0 and 10.0% inhibited fresh biomass accumulation disproportionately in pea germs, i.e. 15.8, 33.1 and 54.8%, respectively, on the average. Similar results were reported by Sahoo et al. (2010), who observed a reduction in the dry weight of soybean, maize and rice after treatment with high concentrations of weed extracts. The germs of cv. Pleven 4 showed a low susceptibility to *S. halepense* extracts regarding fresh biomass production as the reduction was 3.5%, and it was followed by cvs. Modus and Mir (23.2 and 23.7%, respectively). Kamerton and K-80 demonstrated a higher susceptibility with respective reduction values of 33.0 and 42.9%. The suppressive effect of the extracts on the root weight of pea was almost twice as strong (average 43.2%) as on stem weight (23.0%).

Table 2. Influence of *Sorghum halepense* extracts on length and fresh biomass accumulation of germs of *Pisum sativum* cultivars

Cultivar	Type of <i>S. halepense</i> extract*	Concentration %	Indicators						
			Root length cm	Stem length cm	Germ length cm	Root weight g	Stem weight g	Germ weight g	
Kamerton	Aboveground biomass	0	9.21	5.90	15.11	0.203	0.162	0.365	
		1.25	7.23	6.82	14.04	0.132	0.232	0.364	
		2.5	6.04	5.38	11.41	0.140	0.157	0.297	
		5.0	3.05	5.15	8.19	0.056	0.153	0.210	
	Roots	10.0	2.32	4.09	6.41	0.050	0.123	0.172	
		1.25	2.56	4.66	7.23	0.119	0.140	0.259	
		2.5	1.75	4.62	6.37	0.104	0.132	0.237	
		5.0	2.00	4.54	6.54	0.098	0.134	0.232	
		Glyans	10.0	1.35	3.39	4.74	0.068	0.119	0.186
			0	7.99	4.49	12.48	0.152	0.138	0.290
Pleven 4	Aboveground biomass	1.25	7.41	5.64	13.05	0.167	0.214	0.381	
		2.5	3.91	4.19	8.10	0.077	0.106	0.183	
		5.0	2.38	3.11	5.49	0.070	0.102	0.172	
		10.0	2.34	3.10	5.44	0.063	0.096	0.159	
	Roots	1.25	2.76	3.71	6.47	0.080	0.120	0.199	
		2.5	2.66	4.41	7.07	0.090	0.160	0.250	
		5.0	2.66	3.52	6.22	0.076	0.133	0.164	
		10.0	1.36	1.74	3.10	0.033	0.054	0.088	
		K 80	0	6.90	10.67	17.57	0.102	0.265	0.245
			1.25	7.38	11.55	18.93	0.136	0.301	0.437
Mir	Aboveground biomass	2.5	3.93	10.58	14.51	0.083	0.233	0.316	
		5.0	3.95	9.61	13.57	0.055	0.228	0.225	
		10.0	2.52	5.18	7.70	0.030	0.095	0.124	
		1.25	3.44	7.97	11.40	0.054	0.164	0.218	
	Roots	2.5	3.50	9.23	12.72	0.055	0.200	0.255	
		5.0	3.37	7.76	11.13	0.044	0.151	0.195	
		10.0	2.08	3.99	6.08	0.030	0.092	0.123	
		0	9.28	4.60	13.88	0.128	0.181	0.309	
		Aboveground biomass	1.25	7.14	4.40	11.54	0.106	0.127	0.234
			2.5	6.77	4.38	11.14	0.104	0.142	0.246
5.0	3.73		3.91	7.64	0.075	0.115	0.190		
10.0	3.44		2.27	5.71	0.055	0.078	0.133		
Modus	Aboveground biomass	1.25	4.11	4.80	8.91	0.059	0.110	0.168	
		2.5	4.01	4.44	8.45	0.072	0.103	0.175	
		5.0	3.33	2.87	6.20	0.076	0.076	0.152	
		10.0	2.27	2.84	5.11	0.045	0.071	0.116	
	Roots	0	5.88	13.55	7.66	0.071	0.173	0.244	
		1.25	7.41	17.62	10.22	0.083	0.195	0.278	
		2.5	5.32	12.73	7.42	0.068	0.166	0.233	
		5.0	5.84	13.21	7.37	0.053	0.161	0.213	
		10.0	3.04	10.40	7.36	0.034	0.083	0.117	
		1.25	3.30	12.61	9.31	0.046	0.160	0.205	
LSD at the 0.05 probability level	Aboveground biomass	2.5	3.37	10.50	7.14	0.062	0.143	0.205	
		5.0	2.65	9.69	7.04	0.031	0.124	0.155	
		10.0	1.97	6.56	4.59	0.023	0.059	0.082	
		0	8.27	5.43	13.70	0.174	0.176	0.176	
	Roots	1.25	6.71	6.97	13.67	0.127	0.215	0.215	
		2.5	4.85	5.28	10.12	0.110	0.174	0.174	
		5.0	3.52	4.16	7.68	0.079	0.113	0.113	
		10.0	2.52	4.58	7.09	0.053	0.109	0.109	
		1.25	3.73	5.72	9.46	0.100	0.171	0.171	
		2.5	3.01	5.16	8.16	0.089	0.119	0.119	
Factor A	5.0	2.77	4.37	7.14	0.090	0.119	0.119		
	10.0	2.01	2.97	4.98	0.065	0.061	0.061		
Factor B		0.1819	0.0000	0.0000	0.0000	0.0050	0.0028		
Factor C		0.0000	0.0063	0.0000	0.0053	0.0022	0.0006		
A×B		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
A×C		0.6808	0.7108	0.8293	0.5849	0.9370	0.8972		
B×C		0.3538	0.4090	0.6531	0.0554	0.5641	0.6947		
A×B×C		0.0000	0.3983	0.0015	0.0026	0.1713	0.0215		
		0.9851	0.9993	0.9984	0.9302	0.9883	0.9595		

*Weed extracts were prepared by Dr. Pl. Serafimov (IFC, Plevna)

A comparison of effects of the water extracts of *S. halepense* on several basic parameters in pea (Figure 2a) showed that the inhibition of primary germ elongation (average 34.0%) was stronger than the inhibition of fresh weight accumulation (26.3%) and seed germination (18.4%). Overall, the cultivars Mir and Pleven 4 were distinctly more tolerant to the allelopathic activity of the weed (Figure 2b). Growing such cultivars may result in less weed-caused damage (Shahrokhi et al., 2011). A low tolerance was shown by cv. K-80, and intermediate by Modus, Glyans and Kamerton. As the experiment was conducted only in Petri dishes, this research, as well as other similar studies,

require pot and field experiments to fully confirm the present results. Consistent with the facts revealed here were the studies of Verma and Rao (2006), who detected differences among six soybean cultivars in their response to the allelopathic activity of water extracts from various weed species. Baličević et al. (2014) found that maize hybrids differed in their susceptibility to water extracts of *Convolvulus arvensis*: germination of the Bc 574 hybrid was inhibited 24.9%, and germination of the OSSK hybrid 50.7%. Ray and Hastings (1992) and Shahrokhi et al. (2011) reported good tolerance of barley cultivars to extracts from *Amaranthus retroflexus* and *Avena fatua*, respectively.

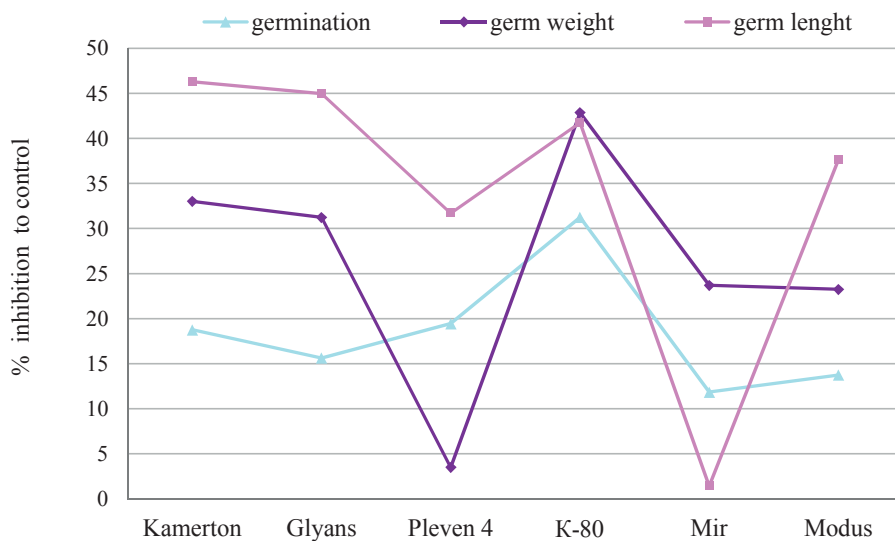


Figure 2a. Inhibitory effects of *S. halepense* extracts on seed germination and initial development of the germ of *P. sativum* cultivars

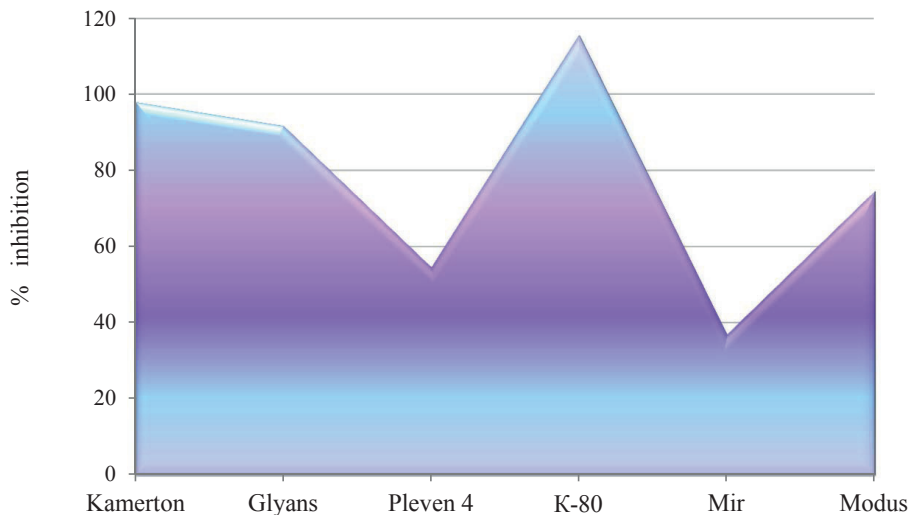


Figure 2b. Total inhibitory effects of *S. halepense* extracts on *P. sativum* cultivars

A data correlation analysis revealed a positive correlation between seed size and the inhibitory effect of weed extracts ($r = 0.744$), which is only logical since larger seeds have greater surface and consequently more contact with the extracts. Shang and Xu (2012) proposed an opposite view, namely that small seeds are subjected to allelopathic impact much more, so that even lower concentrations lead to immediate negative effects. A biochemical analysis of seeds in our previous studies had indicated a negative correlation of $r = -0.425$ on average between the inhibitory influence of weed extracts and protein content in the seeds of different pea cultivars (Georgieva et al., 2008). According to Filippovich (1999) one of the characteristic physical properties of proteins is their ability to absorb on their surface the molecules of organic compounds and ions (this explains their transport function in the plant: some proteins are good carriers of metabolic products). Therefore, the cultivars with higher protein contents in grain were less affected by the depressing effect of *S. halepense* extracts in our present experiment.

CONCLUSIONS

The six studied pea cultivars revealed different levels of susceptibility to the allelopathic impact of root and aboveground biomass extracts of *S. halepense*, which lead to the following important conclusions:

- Growth parameters (length and weight) of the root demonstrated a greater susceptibility to the action of weed extracts in the tested pea cultivars than the corresponding stem parameters

- The extracts' inhibitory effects on *P. sativum* germ length ranged from 1.4 (cv. Mir) to 45.0 % (Kamerton), while the effect on germ weight ranged from 3.5% (Pleven 4) to 42.9% (K-80), and on seed germination from 11.8 (Mir) to 31.3% (K-80). Overall inhibitory effects of *S. halepense* extracts on all studied indicators on *P. sativum* revealed a distinctly higher tolerance of the cultivars Mir and Pleven 10. Low tolerance was manifested by the cultivar K-80, and intermediate by Modus, Glyans and Kamerton.

- The cultivars with larger seed size or lower protein content in grain were more affected by the depressing effect of *S. halepense* extracts.

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Alelopataska otpornost različitih sorti graška na ekstrakte *Sorghum halepense* L. (Pers.)

REZIME

U laboratorijskom eksperimentu ocenjeno je alelopatasko delovanje ekstrakata *Sorghum halepense* na klijanje i početni rast šest sorti graška (*Pisum sativum* subsp. *sativum* i *Pisum sativum* subsp. *arvense*) radi utvrđivanja otpornih sorti. Ispitivane sorte su pokazale različitu osetljivost na alelopatasko delovanje ekstrakata korena i nadzemne biomase *S. halepense*. Parametri rasta korena (dužina i težina) ispitivanih sorti graška pokazali su veću osetljivost na ekstrakte tog korova nego parametri stabla. Inhibitorno delovanje ekstrakata na dužinu klice *P. sativum* kretalo se od 1.4% (sorta Mir) do 45.0% (Kamerton), dok je delovanje na težinu klice bilo od 3.5% (Pleven 4) do 42.9% (K-80), a na klijanje semena od 11.8% (Mir) do 31.3% (K-80). Ukupno inhibitorno delovanje, tj. uticaj ekstrakata *S. halepense* na ispitivane parametre *P. sativum*, pokazalo je da su sorte Mir i Pleven 4 najotpornije. Uzgajanje tih sorti može umanjiti štete od korova. Nisku otpornost je pokazala sorta K-80, a umerenu Modus, Glyans i Kamerton. Sorte krupnog semena i sa niskim sadržajem proteina bile su pod jačim inhibitornim delovanjem ekstrakata *S. halepense*.

Ključne reči: Alelopatija; *Sorghum halepense*; Grašak; Klijanje; Rast