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RESISTANCE OF WEED SPECIES *CHENOPODIUM ALBUM* L. TO ALS-INHIBITORS

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ABSTRACT

The paper presents results of the study conducted in the period 2011-2013 on occurrence of *Chenopodium album* L. resistance to herbicide nicosulfuron, an *acetolactate synthesis* (*ALS*) inhibiting herbicide. In conditions of intensive agricultural production, herbicide weed resistance is a growing problem. Repeated use of herbicides with identical action mechanisms leads to selection of resistant weeds, and elimination of the susceptible biotypes of various species. Herbicide use has provided safety in weed control to agricultural producers, by which other weed control measures have been pushed into the background and greatly reduced. Until now, 415 cases of resistance occurrence have been recorded; of which 212 refers to dicotyledonous weed species, and 203 monocotyledonous weeds. Currently in the world, resistance to ALS inhibitors has been found in 137 weed species, of which 83 belongs to dicotyledonous and 54 to monocotyledonous weeds. At localities in Vojvodina (Bački Maglić and Zmajevo) resistance of *Chenopodium album* L. was tested to the active ingredient nicosulfuron. In the study herbicide nicosulfuron rates of 20; 30; 40; 50 and 100 g of active ingredient per hectare were used. The lowest possible average hypocotyls length at localities Bački Maglić and Zmajevo was 2.55 mm, and 0.71 mm, respectively. The lowest possilbe epicotyls length at localities Bački Maglić and Zmajevo was 2.72 mm, and 0.77 mm, respectively. No statistically significant difference in hypocotyls and epicotyls lengths between seeds of weed species *Chenopodium album* L. from locality Bački Maglić, treated with nicosulfuron and non-treated control, was found, indicating this weed resistance to ALS-inhibitors. So far, in this region resistance to ALS-inhibitors has been confirmed for the following weed species: Amaranthus retroflexus L., Echinochloa crus-galli L. and Datura stramonium L.

Key words: weeds, herbicide resistance, *Chenopodium album* L., nicosulfuron.

INTRODUCTION

Spread of weed species represents one of the biggest concerns in agriculture, especially due to the fact that number of herbicide resistant populations continuously increases (Stankiewicz et al., 2001). In contemporary agriculture, herbicides are dominant remedies for efficient control of majority weeds, but they are not unique solution for complex challenges that nowadays weeds meet. Excessive herbicide use led to fast evolution of weeds, by gaining herbicide resistance (HR) (Beckie, 2006; Powles and Yu, 2010; Heap, 2014). Weed resistance to herbicides is serious and raising agricultural problem in many agricultural systems all over the world. The main research efforts in this field are directed to development of economically available strategies for prevention of resistance occurrence and their monitoring. By their nature weeds have a diverse genetic background that provides them the ability to adapt to many environments. Use of lower or sub lethal rates of herbicides through several generation results in switch of weed population to higher levels of tolerance (Manalil et al., 2011; Norsworthy, 2012). Herbicide site of action is on the one or several places in the plant. At these places may be enzyme proteins, non-enzymatic proteins, or places for cell division. One example is the group of ALS herbicides that inhibit the synthesis of the branched amino acids. These are herbicides, such as sulfonylurea, imidazolinone, triazolopyrimidine. Herbicide resistance is exclusively technical problem that can be overcomed by timely application of appropriate herbicides or by adjusting recommended amounts of herbicide application. Inadequate human activities may lead to the expansion of

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certain weed species that are harmful to humans (Ozair, 2008). Any method of reduction of the selection pressure that leads to the occurrence and development of resistance will also reduce resistance evolution (Konstantinović et al., 2008). Integral approach to weed control has proved as the most efficient (physical, chemical and biological measures), without excessive reliance to any of these methods (Konstantinović and Meseldžija, 2002). Herbicide resistance is obvious example of fast adaptation to human activity, by action of natural selection. Herbicide resistance can be defined as „the inherited ability of weeds to survive herbicide rates that would normally result in efficient control" (Konstantinović, 2011). Resistance to ALS inhibiting herbicides was first discovered in 1984 in Australia for weed species *L. rigidum*, shortly after introduction of the herbicide chlorsulfuron, the first from the group of sulfonylureas. Majority of cases of resistance to ALS herbicides includes modified ALS enzymes with the reduced possibility of herbicide binding to ALS enzyme (Tranel et al., 2012). Some studies suggest that the most important factor leading to the development of resistance to herbicides is over-reliance on herbicides with the same mechanism of action, without using other possibilities of weed control (Norsworthy et al., 2012). The paper of Beckie (2006) proves that weed resistance to ALS-inhibiting herbicides can occur due to use of the identical action mechanism with at least five applications. Woodyard et al. (2009) established synergistic joint action between HPPD inhibitor and PS II inhibitors to (*Chenopodium album* L.) and (*Ambrosia trifida* L.) in a maize crop. It may occur as a result of: reduction in the absorption and translocation of herbicide in the plant organism, the intensification of the process of decomposition and reduction of herbicide activation in plant organism and the specificity of physiological processes and their changes under the influence of herbicides (Janjić, 1997; Konstantinović et al., 2000; Moss, 2002). Research efforts in this area are focused on developing of a strategy for prevention of resistance occurrence (Neve, 2007). Plant resistance is divided into three groups: resistant to lethal rates of herbicides, herbicide tolerant and resistant to herbicides (Răducanu, 2004).

MATERIAL AND METHODS

In the period 2011-2013 weed species *Chenopodium album* L. was studied in laboratory conditions to resistance occurrence to ALS-inhibiting herbicides. Seeds of the studied biotypes of this weed species were collected from different localities of Vojvodina with a long history of ALS-inhibiting herbicide use (2008-2013). Field monitoring was also included in this method. More indicators of resistance occurrence in the field had been established such as: efficiency in control of other susceptible species, presence of plants that survived herbicide application beside those wilted, past experiences, history of herbicide use (Moss, 1995). Populations from ruderal sites were used as a susceptible standard. The herbicide was applied to the collected seeds of *Chenopodium album* L. in a range of rates of 20; 30; 40; 50; 100; g a.i. of nicosulfuron/ha; control remained untreated. The applied herbicide rates represent lower values than minimal prescribed herbicide rates of 20 and 30 g a.i./ha, minimum (40 g a.i./ha) and maximum (50 g a.i./ha) rate of herbicide efficiency, as well as phytotoxic rate of 100 g a.i./ha of the studied herbicide nicosulfuron. We studied the extent to which the occurred resistance was determined through morphological parameters such as the length of the epicotyls and hypocotyls. The study involved monitoring of the history of herbicide application at the studied localities, and collecting seeds of *Chenopodium album* L. from 40 randomly chosen plants of the same biotype of the studied weed species. After measuring and systematization, data were statistically processed. Statistical data processing was done by analysis of variance (ANOVA); significant differences were evaluated by t-test.

Seed was collected from August to October from plots with long history of ALS herbicides use (Table 1). The collected seeds were dried at 25 ºC for five days, and then

exposed to the temperature of 17 ºC and to 54 % of air humidity until setting of the trial (Adkins et al., 1997). Seeds placed in Petri dishes were germinated in controlled conditions of climate chamber. Night and day regime in climate chamber was 16 h/8 h with fluorescent lamps of 83 μE m^{-2} s^{-1} photon flux density, daily temperature 18 ºC and 10 ºC night and 85/54 maximum and minimum of relative air humidity Each Petri dish contained 30 seeds. Before setting of the trial *Chenopodium album* L. seeds were treated with 2% of the product Benomil (500 g a.i. benomyl/kg), for the purpose of seeds disinfection. Results of germination, as well as seed hypocotyls and epicotyls lengths were obtained after measuring 25 days after setting of the trial, i.e. treatment of seeds of the studied weed species by herbicide nicosulfuron.

Table 1. History of herbicides used in treatments of fields from which *Chenopodium album* seed samples were collected at localities Bački Maglić and Zmajevo

Locality	Year	Crop	Herbicide	Rate
Zmajevo	2008	Soybean	Imazethapyr + Oxasulfuron	70 g a.i./ha+70 g a.i./ha
	2009	Maize	Rimsulfuron	10 g a.i./ha
	2010	Soybean	Imazethapyr	100 g a.i./ha
	2011	Wheat	Tribenuron methyl	15 g a.i. /ha
	2012	Sunflower	Prometrin	1000 g a.i./ha
	2013	***Sobean***	Imazethapyr + Bentazone	40 g a.i./ha + 760 g a.i./ha
B.Maglić	2008	Maize	Rimsulfuron + Dicamba	15g a.i./ha+360 g a.i./ha
	2009	Sugar beet	Triflusulfuron methyl + metamitron	2x15ga.i./ha+1,1kg a.i./ha
	2010	Wheat	Tribenuron methyl	14 g a.i./ha
	2011	Soybean	Imazethapyr	100 g a.i./ha
	2012	Wheat	2,4-D	860 g. a.i./ha
	2013	***Maize***	Nicosulfuron	40 g a.i./ha

Seeds were collected in Vojvodina, at localities Bački Maglić and Zmajevo, and for susceptible populations, as control value seeds were collected from ruderal sites that had never been treated with herbicides. All seeds of the tested weed species were collected manually from the studied localities. After herbicide treatments during vegetation, seeds were collected from 4 plots at each locality with high presence of *Chenopodium album* L. populations.

RESULTS

After evaluation of seed germination, as well as hypocotyls and epicotyls lengths of *Chenopodium album* L. seeds collected at localities Bački Maglić and Zmajevo, t-testing of the obtained results was accomplished. Regarding the number of germinated seeds of *Chenopodium album* L. susceptible population (control) and treated seeds collected at locality Bački Maglić, no statistically significant difference at a confidence level 0.05 was found with the sample size of 100 seeds, each with four replications per treatment (Table 2). In Table 2, Column VII are presented data on the total number of seeds germinated 14 days after storing in controlled conditions of climate chamber.

Testing of the existence of statistical differences in the number of germinated seeds in 14 days time period, with determination of the number every two days, also enabled also observation of treated and untreated seeds of *Chenopodium album* L. dynamics of development. In comparison with the control value in all replications within treatment there were no statistically

significant differences. Lack of significance indicates that the herbicide efficiency in treatments was equaled with the control value and that the herbicide showed no effect on germination of *Chenopodium album* L. seed (Table 2). From data presented in the table, it is obvious that after two days the initial rate of emergence was higher in control than in the treatment, whereas on the sixth and eighth day the number of germinated seeds of *Chenopodium album* L. was almost equaled. A total number of germinated seeds originating from locality Bački Maglić, in comparison with the total number of germinated seeds collected at locality Zmajevo differed significantly. On average, 22.25% of the seeds collected from locality Bački Maglić germinated, and those originating from locality Zmajevo had rate of emergence of 16.25%.

Table 2. Germination of *Chenopodium album* L. seeds collected at locality Bački Maglić

Concentration	I	II	III	IV	V	VI	VII	Diff.	Significance of difference from control
I1 (20 g a.i./ha)	0	5	10	10	16	17	19	0.76	NO
I2	2	4	7	9	10	10	12	0.48	NO
I3	0	2	7	12	16	16	18	0.72	NO
I4	0	5	11	12	13	13	14	0.56	NO
II1 (30 g a.i./ha)	0	3	5	9	12	12	13	0.52	NO
II2	0	2	6	8	7	12	13	0.52	NO
II3	0	1	7	9	10	13	13	0.52	NO
II4	0	2	3	5	6	8	11	0.32	NO
III1(40 g a.i./ha)	0	4	11	14	16	16	17	0.68	NO
III2	0	0	4	8	9	10	14	0.56	NO
III3	0	2	10	13	13	13	13	0.52	NO
III4	0	1	1	6	9	11	13	0.52	NO
IV1(50 g a.i./ha)	0	2	9	12	13	15	15	0.6	NO
IV2	0	3	6	6	10	13	15	0.6	NO
IV3	0	1	4	10	13	17	17	0.68	NO
IV4	0	2	7	10	16	17	17	0.68	NO
V1(100 g a.i./ha)	0	1	2	5	8	12	12	0.48	NO
V2	0	1	1	5	12	16	17	0.68	NO
V3	0	2	3	9	12	13	13	0.52	NO
V4	0	1	2	2	6	13	13	0.52	NO
VI1 Control	7	8	13	13	13	12	17	0.68	
VI2	6	6	12	15	16	10	17	0.68	
VI3	5	7	12	17	10	14	16	0.64	
VI4	5	10	12	14	10	10	15	0.6	

(I1; I2; I3; I4 – replications within one studied concentration).
Diff – deviation from mean values of replications I-VII.

Results of bioassays with seed samples from locality Zmajevo tested in all concentrations of the herbicide nicosulfuron showed the existence of statistically significant differences on the level of confidence 0.005 (Table 3). These results indicate that herbicide efficiency with seed samples for the studied weed species from this location was still good.

Table 3. Germination of *Chenopodium album* L. seed collected at locality Zmajevo

Concentration	I	II	III	IV	V	VI	VII	Diff.	Significance of difference from control
I1 (20 g a.i./ha)	0	7	11	12	14	13	16	0.64	YES
I2	1	9	9	10	10	11	11	0.44	YES
I3	1	5	11	17	19	20	20	0.8	YES
I4	0	6	9	11	12	15	17	0.68	YES
II1 (30 g a.i./ha)	0	3	8	14	15	16	18	0.72	YES
II2	1	6	10	12	13	16	17	0.68	YES
II3	1	9	10	13	13	14	15	0.6	YES
II4	0	9	13	15	15	16	17	0.68	YES
III1(40 g a.i./ha)	0	2	8	10	11	15	13	0.52	YES
III2	0	3	6	9	12	15	16	0.64	YES
III3	1	8	5	11	13	14	15	0.6	YES
III4	0	5	6	8	11	13	15	0.6	YES
IV1(50 g a.i./ha)	0	5	6	6	8	11	12	0.48	YES
IV2	0	2	9	13	14	15	16	0.64	YES
IV3	0	4	7	12	12	13	14	0.56	YES
IV4	0	8	11	12	15	17	17	0.68	YES
V1(100 g a.i./ha)	0	2	6	8	9	9	10	0.4	YES
V2	1	4	6	7	7	7	11	0.44	YES
V3	0	7	8	9	10	10	10	0.4	YES
V4	0	1	4	5	7	9	13	0.52	YES
VI1 Control	9	13	16	18	20	22	25	1	
VI2	5	9	15	19	22	23	23	0.92	
VI3	8	14	18	21	24	24	25	1	
VI4	6	9	17	20	21	23	25	1	

(I1; I2; I3; I4 – replications within one studied concentration).
Diff. – deviation from mean values of replications I-VII.

Testing of statistical difference in values hypocotyls and epicotyls length seeds collected at locality Bački Maglić revealed that in comparison to the control, there was no statistically significant difference (Tables 4 and 5).

Based upon *P*-values in Table 4 are noticeable statistically significant differences in epicotyls lengths at level of confidence 0.005. In Table 4 it can also be observed that there were differences in hypocotyls length of *Chenopodium album* L. seed from locality Bački Maglić. In the period 2011-2013 the average epicotyls length of seeds from locality Bački Maglić ranged from minimal value of 2.72 mm up to 7.52 mm. Measured hypocotyls length of seeds from the same locality also ranged from 2.55 mm up to 16.015 mm.

Based upon P-values in Table 5 are noticeable statistically significant differences in epicotyls lengths at level of confidence 0.005. In Table 4 it can also be observed that there were differences in hypocotyls length of *Chenopodium album* L. seed from locality Zmajevo. In the period 2011-2013 the average value of epicotyls length of seeds from locality Zmajevo ranged from minimal value 0.775 mm up to 3.030 mm. The length of hypocotyls of seeds from locality Zmajevo also ranged from 0.710 mm up to 1.330 mm.

Table 4. Values of epicotyls and hypocotyls length of *Chenopodium album* L. seed collected at locality Bački Maglić

Variable	T-test							
	Mean	Std.Dv.	N	Diff.	Std.Dv.Diff.	t	df	p
EP05BM	7.035000	6.865819						
EPKBM	7.580000	5.169839	200	-0.54500	7.463679	-1.0327	199	0.303015
EP08BM	7.525000	7.152283						
EPKBM	7.580000	5.169839	200	-0.05500	6.994037	-0.1112	199	0.911561
EP1BM	6.510000	5.516526						
EPKBM	7.580000	5.169839	200	-1.07000	6.471049	-2,3384	199	0.020358
EP12BM	6.940000	5.784783						
EPKBM	7.580000	5.169839	200	-0.64000	5.774698	-1.5673	199	0.118622
EP24BM	2.720000	3.247202						
EPKBM	7.580000	5.169839	200	-4.86000	5.663105	-12.1366	199	0.000000
HP05BM	16.01500	9.963490						
HPKBM	17.31500	8.722165	200	-1.3000	10.33460	-1.7790	199	0.076774
HP08BM	15.84000	9.095496						
HPKBM	17.31500	8.722165	200	-1.4750	8.62621	-2.4182	199	0.016499
HP1BM	15.34000	9.266268						
HPKBM	17.31500	8.722165	200	-1.9750	9.92608	-2.8139	199	0.005386
HP12BM	14.73000	8.233838						
HPKBM	17.31500	8.722165	200	-2.5850	7.27029	-5.0283	199	0.000001
HP24BM	2.55000	2.667033						
HPKBM	17.31500	8.722165	200	-14.7650	9.16020	-22.7952	199	0.000000

EP05BM (EP-epicotyl; HP-hypocotyl; 05 was 20 g a.i./ha; 08 is 30 g a.i./ha; 1 is 40 g a.i./ha; 12 is 50 g a.i./ha; 24 100 g a.i./ha; K is control and BM-locality Bački Maglić).

*Table 5.*Values of epicotyls and hypocotyls length of *Chenopodium album* L. seed collected at locality Zmajevo

Variable	T-test							
	Mean	Std.Dv.	N	Diff.	Std. Dv.Diff.	t	df	p
EP05BM	3.030000	1.920296						
EPKZM	9.380000	6.645027	200	-6.35000	6.044849	-14.8560	199	0.303015
EP08ZM	1.885000	1.560110						
EPKZM	9.380000	6.645027	200	-7.49500	6.161966	-17.2015	199	0.911561
EP1ZM	1.865000	2.481444						
EPKZM	9.380000	6.645027	200	-7.51500	6.455084	-16.4643	199	0.020358
EP12ZM	1.325000	1.399704						
EPKZM	9.380000	6.645027	200	-8.05500	6.312783	-18.0451	199	0.118622
EP24ZM	0.775000	1.072439						
EPKZM	9.380000	6.645027	200	-8.60500	6.383787	-19.0628	199	0.000000
HP05ZM	1.24000	0.84020						
HPKZM	23.24000	12.76193	200	-22.0000	12.45252	-24.9851	199	0.000027
HP08ZM	1.33000	0.94635						
HPKZM	23.24000	12.76193	200	-21.9100	12.56748	-24.6552	199	0.000000
HP1ZM	1.15500	1.52070						
HPKZM	23.24000	12.76193	200	-22.0850	12.49594	-24.9944	199	0.000000
HP12ZM	0.93500	1.11670						
HPKZM	23.24000	12.76193	200	-22.3050	12.42513	-25.3873	199	0.000000
HP24ZM	0.71000	0.83027						
HPKZM	23.24000	12.76193	200	-22.5300	12.60051	-25.2865	199	0.000000

EP05ZM (EP-epicotyl; HP-hypocotyl; 05 was 20 g a.i./ha; 08 is 30 g a.i./ha; 1 is 40 g a.i./ha; 12 is 50 g a.i./ha; 24 100 g a.i./ha; K is control and ZM-locality Zmajevo).

At locality Bački Maglić, a statistically significant difference was calculated between high rates of herbicide application of 2.4 l/ha and control. At locality Zmajevo there were statistically significant differences from control in epicotyls and hypocotyls length of the studied *Chenopodium album* L. seeds in comparison to all studied concentrations rates. Values of hypocotyls and epicotyls length of *Chenopodium album* L. seed in the control, at locality Zmajevo were higher in comparison to the control values at locality Bački Maglić. At locality Bački Maglić values of hypocotyls length obtained after the application of the lowest herbicide rate of 0.5 l/ha slightly decreased as compared to the applied herbicide at a rate of 2.4 l/ha, at which statistically significantly lower value in hypocotyls length was recorded. Value of epicotyls length at locality Bački Maglić did not decrease proportionally with the increase in the applied herbicide rates, but it was of almost equal values as the control up to the concentration of 2.4 l/ha, at which statistically significant difference was recorded in comparison to the control (Tables 4 and 5). Figure 1 presents values of hypocotyls and epicotyls length for both of the studied localities. Overall low value for hypocotyls and epicotyls length of *Chenopodium album* L. seed in control was measured at locality Bački Maglić in comparison to locality Zmajevo.

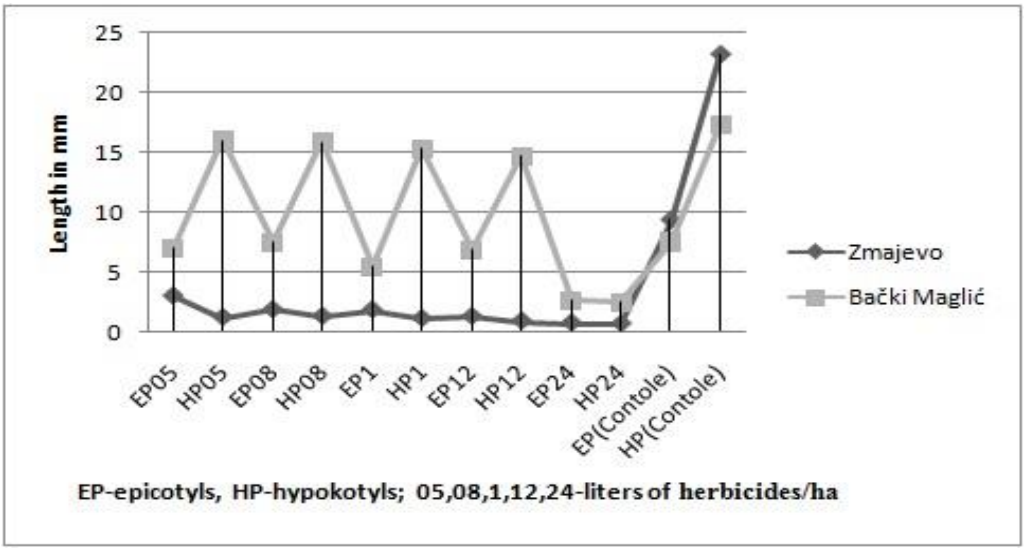

Figure 1. Distribution of hypocotyls and epicotyls length of seeds from localities Zmajevo and Bački Maglić

DISCUSSION

Three years of the study of weed species *Chenopodium album* L. to resistance to acetolactate synthesis (ALS) inhibiting herbicide, provided preliminary test results in laboratory conditions. Low herbicide efficiency in the field certainly can be ascribed to numerous factors. At locations (Bački Maglić, Zmajevo) with several years lasting use of acetolactate synthesis (ALS) inhibiting herbicides, changes of *Chenopodium album* L. population in field were established, which were also confirmed by laboratory bioassays for establishment of resistance. Population of *Chenopodium album* L. at locality Bački Maglić was tolerant to the treatment by herbicides belonging to HRAC group B, which was visually established in the field as incomplete drying of the studied weed species. Based upon results obtained by biological studies of the treated *Chenopodium album* L., these populations differ in their susceptibility to herbicide nicosulfuron in comparison to the susceptible standard *Chenopodium album* L., collected at ruderal sites. Morphometric parameters were analyzed in many methods applied for weed resistance to herbicides from different chemical groups (Beckie et

al., 2000; Hanson et al., 2004; Corbett and Tardif, 2006). For weed species *Chenopodium album* L., seeds collected at locality Zmajevo did not show presence of resistance, as statistically significant difference in hypocotyls and epicotyls length in comparison to the control value did not exist. It is assumed that herbicide keeps controlling weed species *Chenopodium album* L., because the treated seeds of the studied weed species differed significantly in comparison to the control. Results of bioassays on seed samples collected at locality B. Maglic showed that there was statistically significant difference between hyypocotyls and epicotyls length of *Chenopodium album* L. seed treated by the herbicide nicosulfuron at a rate of 2.4 l/ha, in comparison to the control. The lowest recorded medium values of hypocotyls and epicotyls lengths of *Chenopodium album* L. populations were at concentration of 100 g a.i./ha for seeds from localities Bački Maglić and Zmajevo. Mean value of *Chenopodium album* L. seed epicotyls at both localities was 1.747 mm, and medium length of *Chenopodium album* L. seed hypocotyls was 1.63 mm. Medium length of seed epicotyls of the studied weed species in control, at both localities was 8.48 mm and hypocotyls medium length was 20.277 mm. Establishment of resistance based upon morphometric change is less precise method than the method of ALS enzyme activity study, but the results suggest that population *Chenopodium album* L. at locality Bački Maglić was less susceptible to the herbicide nicosulfuron. Results can also be associated with the higher or lower activity of ALS enzymes, which can be the consequence of decreased or increased action of the herbicide nicosulfuron. These conclusions have been presented in many studies in which the method of ALS enzymes activity was applied in the population studies (Saari et al., 1994; Lovell et al., 1996; Sprague et al., 1997). However, results obtained in this study suggest that resistance of *Chenopodium album* L. to the studied herbicide nicosulfuron exists. Moreover, our studies carried out in the period 2005-2006 at various localities such as Krivaja, Sava Kovačević, Kikinda and Bečej confirmed resistance to the herbicide nicosulfuron, as well as to the herbicide imazethapyr (Konstantinović et al., 2007).

CONCLUSIONS

At locality Bački Maglić, there were no statistically significant differences in hypocotyls and epicotyls length for all of the applied herbicide rates, excepting rate of 1.2 l/ha, for which there was statistically significant difference. Non-existence of statistically significant differences in germination of *Chenopodium album* L. seed was also established for all applied nicosulfuron rates of 0.5, 0.8, 1.0, 1.2 and 2.4 l/ha. Establishment of non-existence of statistically significant differences between treatments and control in laboratory conditions from samples taken out at locality Bački Maglić, led to conclusion that there exists resistance to the herbicide nicosulfuron. At locality Zmajevo, statistically significant difference was determined in epicotyls and hypocotyls length of weed species *Chenopodium album* L. seed, as well as in abundance of germinated seeds, and therefore resistance for the studied herbicide cannot be confirmed.

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