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ALLELOPATHIC EFFECT OF WEED SPECIES *AMARANTHUS RETROFLEXUS* L. ON MAIZE SEED GERMINATION

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ABSTRACT

In order to examine allelopathic effect of *Amaranthus retroflexus* L., on germination capability and epicotyls and hypocotyls growth of maize (*Zea mays* L.) seeds a study was performed in laboratory conditions. The studies were conducted in the period 2011-2012 at Faculty of Agriculture in Novi Sad. Treatments were set up in randomised block design in four replications for each of four concentration ranges of 25, 50, 75 and 100 g l^{-1} of aqueous extract made of leaves and stem (above-ground parts) and 4 identical extract concentrations made of root (underground part) of weed species *A. retroflexus* L. Control variant was maize seed treated by distilled water. During the studies epicotyls and hypocotyls of maize seeds were measured after treatments with different concentrations of extracts made of underground and above-ground parts of *A. retroflexus* L., and the obtained values were compared with the control. The obtained results suggest that the extract from the above-ground parts of *A. retroflexus* L. in low concentrations of 25 g l^{-1} and 50 g l^{-1} had inhibiting effect to the growth of maize hypocotyls length, while having no statistically significant effect on epicotyls in all concentration, except the extract concentration of 100 g l^{-1}, for which statistically significant effect on epicotyls growth was recorded. The extract from underground part of *A. retroflexus* L. had inhibiting effect to growth of maize epicotyls at all concentrations, while it had no effect to hypocotyls growth at any of the tested concentrations.

Key words: allelopathy, *Amaranthus retroflexus (*L.*)*, aqueous extract, maize, seed germination.

INTRODUCTION

Plants can release harmful chemical substances to the environment reducing growth and establishment of other plants in their vicinity. The process is known as allelopathy. However, chemical substances with allelopathic properties have also other ecological roles, such as plant defence and regulation of soil biotype that has impact to soil degradation or fertility. These ecosystem-scale roles of allelopathic chemicals can augment, attenuate or modify their community-scale functions (Inderjit et al., 2011).

In allelopathy research, inhibitory substances are often argued to explain growth pattern, while other substances remain neglected (Inderjit and Nilsen, 2003). Leachate may interfere with plant seedling growth by (i) causing plant growth inhibition (allelopathy), (ii) causing N immobilization, and/or (iii) enhancing microbial population that can out complete plant seedlings (Northup et al., 1995; Schmidt and Ley, 1999; Inderjit, 2006).

In the last ten years, allelopathy has been intensively studied, with the aim of production of biofertilizers and bioherbicides, and many researchers analysed effects of aqueous extracts made of different plant parts to growth and development of other species (Kazinczi et al., 2004; Paul and Sultana, 2004; Uremis et al., 2005; Javaid et al., 2006; Xingsiang et al., 2009; Qian et al., 2010).

Weeds have harmful effect to crops due to release of phytotoxins from their seeds, or as the result of decomposition of plant remains (Narwal, 2004). Plant parts that include weed leaf, stem, root and fruit can have allelopathic potential (Alam and Islam, 2002; Tinnin and Muller, 2006). Leaves are main parts for production of allelopathic substances and their action is maximal, while root possesses minimal quantities of allelopathic substances (Sisodia and Siddiqui,

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2010; Rice, 1984). Various parts of the same weed species have different allelopathic activity and differ in capability of harmful action to germination capability and to beginning growth stages of cultivated crops (Aziz et al., 2008). Allelopathic effect of weeds that influence germination capability and growth of seedlings of the crop also differs from weed to weed (Hamayun et al., 2005). Interaction between weeds and cultivated plants is simultaneous and/or subsequent with direct or indirect action of one plant species to another. The synthesis of various chemical compounds – allelochemicals – that plants release to the environment, create the inhibitory and/or stimulant effect on germination of seeds and development of crops (Verma & Rao, 2006;. Aleksieva & Serafimov, 2008).

A. retroflexus L. is one of the most important weed species in numerous agricultural areas, being the third widespread dicotyledonous weed species in the world (Shimi and Termeh, 2004). *A. retroflexus*, reduced sugar beet yield by 12% and 31% with density of 1.5 plants per m^2 (Stebbing et al., 2000). Yarnia et al. (2009) also established that allelopathic activity of populations of *A. retroflexus* can vary by 50 to 60 percent by preparing extract from different plant parts.

MATERIAL AND METHODS

In the period 2011-2012, at locality Kać near Novi Sad plant parts of *A. retroflexus* L. were collected. Plant material was divided into above-ground parts, i.e. stem plus leaf and underground part, i. e. root. Underground and above-ground parts were pulverized, after which separate extracts were made from each of these parts of *A. retroflexus* L.

Extract of above-ground and underground part of *A.retroflexus* L. was made in a range of concentrations of 25, 50, 75, and 100 g per litre of distilled water. Filter paper in Petri dishes, of 150 x 25 mm in size was moistened by 8 ml of the obtained extracts, and tested maize seed was germinated on it. Control was moistened by distilled water. Maize seed surface was sterilized before adding of extracts according to Elemar and Filho (2005). Each concentration of *A. retroflexus* L. extracts was made in four replications. Samples were laid in thermostatic device at 22 ± 2°C for 7 days. Laboratory tests were set up in randomised block design in four replications. Each Petri dish contained 25 maize seeds, i.e. 100 seeds per treatment. All measurements were performed on daily basis, five days after moistening of seeds of the tested crop. Existence of allelopathic activity of the studied weed species on maize crop was established by measurement of crop seed epicotyls and hypocotyls lengths in mm (Marinov-Serafimova et al., 2007).

The aim of the study was to establish if there exist allelopathic relations between maize hypocotyls and epicotyls growth after treatment with above-ground, i.e. underground part of *A. retroflexus* L.

RESULTS

Based upon conducted studies, data on effect of extracts made of underground and above-ground parts of *A. retroflexus* L. to hypocotyls and epicotyls length of maize seed were obtained. Extracts made of above-ground parts in concentrations of 50, 75 and 100 g l^{-1}, showed significant effect on maize hypocotyls growth. Estimated possibilities of the error of the first grade for the extract made of above-ground parts in concentration of 50 g l^{-1} was 0.00022, for concentration of 75 g l^{-1} it was 0.017, and for concentration of 100 g l^{-1} it measured 0.0018. The obtained results for extract concentration of 25 g l^{-1} made of above-ground parts of *A. retroflexus* L. showed that hypocotyls length for was 27.5 mm, which was less in comparison to the value of control of 28.96 mm; for the medium extract concentration of 50 g l^{-1} determined hypocotyls length was 26.86 mm, and control was 29.18 mm. At higher concentrations were also established lower values of the average hypocotyls lengths, i.e. for extract concentration of 75 g l^{-1} hypocotyls length was 26.53 mm, whereas in the highest concentration of 100 g l^{-1} maize seed hypocotyls length was the lowest, i. e. 24.7 mm in comparison to control variant that was

28.9 mm. Measured values of maize seed hypocotyls treated by extract of the above-ground parts of *A. retroflexus* L. confirmed that extract showed allelopathic – inhibitory effect to the growth of hypocotyls in comparison to the obtained control values.

The extract made of the above-ground parts of *A. retroflexus* L. in concentration of 100 g l^{-1} showed allelopathic effect to the growth of maize seed epicotyls, while concentrations of 25, 50 and 75 g l^{-1} there was no allelopathic effect. For concentration of 25 g l^{-1} of extract made of above-ground parts of *A. retroflexus* L., the average value of epicotyls length was 7.23 mm, and control was 8.29 mm, for concentration of extract of 50 g l^{-1} the established value of epicotyls length was 6.81 mm, while control was 8.09 mm, and concentration of 75 g l^{-1} resulted in epicotyls length of 6.98 mm, with control value of 7.81 mm. T-test showed statistically significant difference (P=0.0018) between the lengths of maize seed epicotyls only in the case of the extract made of the above-ground parts of *A. retroflexus* L. in concentration of 100 g l^{-1}. Application of extract made of the above-ground parts of *A. retroflexus* L. in concentration of 100 g l^{-1} resulted in maize seed epicotyls length of 5.83 mm, while control value was 8.16 mm (Table 1).

Table 1. Statistical data on effect of the above-ground parts of *Amaranthus retroflexus* L., to germination of maize seed

Extract from above-ground parts of *A. retroflexus* L.	T-test for Dependent Samples. Marked differences are significant at P<0.05			
	Mean	Std.Dev.	Diff.	Std.Dev.Diff
25 g l^{-1} to the growth of hypocotyls	27.5	2.048216		
CONTROL	28.96	1.371694	-1.47	2.289500
50 g l^{-1} to the growth of hypocotyls	26.86	1.334691		
CONTROL	29.18	2.066711	-2.31500	1.916810
75 g l^{-1} to the growth of hypocotyls	26.53	2.496815		
CONTROL	28.88	3.079604	-2.35312	3.519391
100 g l^{-1} to the growth of hypocotyls	24.7	3.559564		
CONTROL	28.9	3.047709	-4.53	4.534794
25 g l^{-1} to the growth of epicotyls	7.23	1.695785		
CONTROL	8.29	2.479671	-1.06625	2.776213
50 g l^{-1} to the growth of epicotyls	6.81	1.914666		
CONTROL	8.09	3.171056	-1.29250	3.828334
75 g l^{-1} to the growth of epicotyls	6.98	2.024147		
CONTROL	7.81	3.292084	-0.998750	3.330355
100 g l^{-1} to the growth of epicotyls	5.83	1.162267		
CONTROL	8.16	2.950447	-2.32	2.5

Bioassay revealed that for extract of the underground part of *A. retroflexus* L. there were no statistically significant deviations between values of maize seed hypocotyls lengths from control values. For variant of 25 g l^{-1} of extract from the underground parts of *A. retroflexus* L. maize seed hypocotyls length was 27.81 mm. while control was 28.96 mm; the application of highest concentration of extract made of underground part of 100 g l^{-1} resulted in average hypocotyls length of 26.75 mm. whereas the average length of hypocotyls in control was 28.9 mm. Length of maize seed hypocotyls in treatment with the extracts made of the underground part of *A. retroflexus* L. did not confirmed allelopathic effect in comparison to the control variant. Extract made of underground part of *A. retroflexus* L. also did not have show allelopathic effect to epicotyls length in extract concentrations of 50 and 75 g l^{-1}, while in concentrations of 25 and 100 g l^{-1} allelopathic effect was recorded. For extract made of underground part of *A. retroflexus* L. in concentration of 25 g l^{-1} established value of maize seed epicotyls was 6.42 mm. and control value was 8.29 mm. for the mean extract concentration of 50 g l^{-1} epicotyls length was 6.42 mm. and control value was 8.09 mm. Values of epicotyls lengths for extract concentration of 75 g l^{-1} was 5.85 mm. and control was 7.81, whereas the highest extract concentration of 100 g l^{-1} resulted in values for epicotyls length of 5.48 mm in comparison to control of 7.68 mm (Table 2).

Table 2. Statistical data on effect of the underground parts of *Amaranthus retroflexus* L., to germination of maize seed

Extract from underground parts of *A. retroflexus* L.	T-test for Dependent Samples. Marked differences are significant at $P<0.05$			
	Mean	Std.Dev.	Diff.	Std.Dev.Diff.
25 g l^{-1} to the growth of *hypocotyls*	27.81	3.462592		
CONTROL	28.96	1.371694	-1.10563	3.608187
50 g l^{-1} to the growth of *hypocotyls*	27.89	2.612752		
CONTROL	29.18	2.066711	-1.28750	3.518832
75 g l^{-1} to the growth of *hypocotyls*	27.18	1.757371		
CONTROL	28.88	3.079604	-1.69812	3.418599
100 g l^{-1} to the growth of *hypocotyls*	26.75	3.363108		
CONTROL	28.9	3.047709	-2.23875	5.241309
25 g l^{-1} to the growth of epicotyls	6.42	2.396191		
CONTROL	8.29	2.479671	-1.87	2.79
50 g l^{-1} to the growth of epicotyls	6.42	2.461563		
CONTROL	8.09	3.171056	-1.82750	4.758135
75 g l^{-1} to the growth of epicotyls	5.85	2.587333		
CONTROL	7.81	3.292084	-2.12688	4.626875
100 g l^{-1} to the growth of epicotyls	5.48	2.363920		
CONTROL	7.68	2.950447	-2.67	4.04

For experimental variants in concentrations of 25 and 100 g l^{-1} of extracts made of underground part of *A. retroflexus* L., statistically significant difference (P=0.017 and P=0.018) was measured in comparison with the control values. Experimental variant of extracts of 50 and 75 g l^{-1} did not show statistically significant differences in comparison to control.

DISCUSSION

In the study allelopathic effect of the weed species *A. retroflexus* L. was established for early growth and development of maize hypocotyls. Extracts made of underground parts of *A. retroflexus* L. showed inhibitory effect to maize seed hypocotyls length. Extract made of the underground parts, in concentration of 100 g l^{-1} significantly affected epicotyls growth, while the lower three concentrations did not show a significant effect. Extracts made of underground parts of *A. retroflexus* L. did not show statistically significant effect to hypocotyls growth in any concentration. Extracts made of underground part of *A. retroflexus* L. in concentrations of 25 and 100 g l^{-1} showed statistically significant effect to the growth of epicotyls, while concentrations of 50 and 75 g l^{-1} had no statistically significant effect.

Based upon studies performed in the period 2011-2012 it can be concluded that extract made of underground parts of *A. retroflexus* L. showed significant allelopathy effect by inhibiting maize seed hypocotyls growth. i. e. the results suggest that increase in concentration causes also increase in difference in hypocotyls lengths. On average, concentration of 25 g l^{-1} of the above-ground extract of *A. retroflexus* L. reduced the value of hypocotyls length by 1.47 mm, while concentration of 100 g l^{-1} had hypocotyls length 4.53 mm lower in comparison to control. Extract of the underground part did not show inhibitory effect to hypocotyls growth. Inhibiting effect to the growth of epicotyls length had extract made of underground parts in the highest concentration of 100 g l^{-1} with the average difference of 2.32 mm in comparison to control value. Extract from the underground part had inhibiting effect with the lowest concentration of 25 g l^{-1} and the lower average epicotyls value of 1.87 mm in relation to control. The highest concentration of 100 g l^{-1} had the highest difference in epicotyls length that was 2.67 mm lower in comparison to control variant. According to the studies of Khan et al. (2011) influence of *Parthenium hysterophorus* L. extract on germination capability and growth of soybean, bean and maize crops suggest that increase in concentrations of *P. hysterophorus* L. extract causes reduction in values of the studied parameters in the above mentioned crops. Studies performed by Waqas et al. (2011) on influence of wheat and chickpea seed germination capability after treatment with extracts of weed species *Cyperus rotundus. Datura alba* and *Silybum marianum* obtained significant results. Statistical analysis of data suggests that different weed extracts have significant influence to dry biomass of the tested species. It was established that increase in extracts concentrations of weed species *C. rotundus, D. alba* and *S. marianum* resulted in reduction of dry biomass of wheat and chickpea. The most (2.006 g) of dry biomass was recorded by extract of *C. rotundus*, followed by *D. alba* (1.9 g), while the minimum (1.0 g) results were obtained for *S. marianum*. The driest mass (2.29 g) was recorded in control. The experimental data obtained during studies of Sipos et al. (2012) confirmed allelopathic effects in a range of solutions of 5, 15, 25, 50, 75 and 100% of aqueous extract made of *Armoracia rusticana* L. root. Extracts showed inhibitory effect to germination capability of *Triticosecale* Witt., *Triticum aestivum* L. and *Hordeum vulgare* L. Extracts had inhibitory effect to germination capability of *Triticosecale* Witt., *T. aestivum* L. and *H. vulgare* L. The highest inhibitory effect to germination of *Triticosecale* Witt., *T. aestivum* L. and *H. vulgare* L. was obtained by extracts in concentrations of 25% and 100%. These studies also suggested that inhibitory allelopathic effect is enhanced with increase in extract concentrations, indicating that 100% concentration also had the highest allelopathic effect to germination capability of *Triticosecale* Witt. *T. aestivum* L. and *H. vulgare* L. Studied extracts of *A. retroflexus* L. also had the highest inhibitory effect to germination capability of maize seed at highest concentration rate of 100%.

CONCLUSION

The applied extracts made of the above-ground parts. i. e. leaf plus stem and underground part, i.e. root of weed species *A. retroflexus* L. inhibited maize seed epicotyls and hypocotyls growth. Experimental data confirm results obtained by other authors (Turk & Tawaha, 2002 and Ashrafi et al., 2007), suggesting that allelopathic action refers to inhibition of germination which is more pronounced in the phase of the growth of the seedlings.

The studies revealed reduced germination capability of *Zea mays* L. seed hypocotyls after treatment by extract made of the above-ground parts of *A. retroflexus* L. which was supported by calculated statistical significance that indicated existence of allelopathic effect. Percentage of hypocotyls lengths in bioassays conducted with extracts made of the above-ground parts of *A. retroflexus* was lower than the control value for 5.04-14.53%.

Extract made of the underground part of *A. retroflexus* L. did not have allelopathic effect to the growth of maize seed hypocotyls. The obtained results confirmed that extract made of the above-ground parts of *A. retroflexus* L. had allelopathic effect to the length of maize seed epicotyls only in concentration of 100 g l^{-1}. Extract made of the underground part of *A. retroflexus* L. showed allelopathic effect to *Zea mays* L. seed epicotyls in concentrations of 25 and 100 g l^{-1} in values of 22.53-28.8% lower in comparison to the control variant.

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