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Short Communication

Influence of winter savory (*Satureja montana* L.) aqueous extract on antioxidant properties of Jimson weed (*Datura stramonium* L.)

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ABSTRACT: Due to an increase in the number of herbicide-resistant weeds and environmental concerns about the use of synthetic herbicides, a great effort is being made in designing alternative weed management strategies. The present study was carried out in order to examine the impact of winter savory (*Satureja montana*) aqueous extract in natural weed management. We evaluated the effect of two concentrations (0.1 and 0.2%) of *S. montana* aqueous extract on the activity of antioxidant enzymes and the lipid peroxidation process in Jimson weed (*Datura stramonium*) seedlings. Our results showed that *S. montana* aqueous extract induced lipid peroxidation in roots of Jimson weed seedlings 72 hours after the treatment.

Keywords: allelopathy, *Datura stramonium*, *Satureja montana*

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Satureja montana L. (Lamiaceae) (winter savory) is a well known aromatic plant, often used in traditional medicine (SILVA *et al.* 2009). The aqueous extract of *S. montana* contains active compounds like the phenolic components caffeic acid and gallic acid (ŠUĆUR *et al.* 2015). As one of the major groups of compounds, plant phenolics possess a broad spectrum of chemical and biological activities (Zeng *et al.* 2001). The application of allelopathic interactions and natural substances in agricultural practice can reduce the use of synthetic pesticides in the management of weeds. Allelopathic interactions are based on the production of secondary biomolecules by plants which exert positive or negative effects on other plants (Macías *et al.* 2004). One of the main effects of released secondary biomolecules on target plants is the excessive production of reactive oxygen species (ROS), molecules very toxic to cells (Bogatek & Gniazdowska 2007). Plants are able to cope with oxidative damage by using some antioxidant enzymes and free radical scavengers. Superoxide dismutase is the first enzyme in the detoxifying process; it catalyses the dismutation of superoxide free radical anions to molecular oxygen and water, preventing the formation of other more toxic oxygen species such as the OH radical. Catalase and peroxidases metabolise hydrogen peroxide. The typical catalase reaction is the dismutation of two molecules of hydrogen peroxide to water and molecular oxygen (Mhamdi *et al.* 2010). Peroxidases catalyse the oxidation of a wide variety of substrates, using H_2O_2 or other peroxides (Hamid & Rehman 2009). Physiological activities are hindered by allelopathy (Siddique & Ismail 2013), and activity of antioxidant enzymes can thus be used as an indicator of oxidative stress in plants (Fakoorziba *et al.* 2014). One of the most resistant weed species is Jimson weed (*Datura stramonium* L.). Jimson weed is a widespread annual plant, encountered in maize and sunflower fields. All parts of the plant are toxic, and the toxins in it are tropane belladonna alkaloids (Binev *et al.* 2006; Maheshwari *et al.* 2013). It inhibits the growth of surrounding plants through the mecha-

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Table 1. Effects of two concentrations (0.1 and 0.2%) of *S. montana* aqueous extract on the activity of antioxidant enzymes (U/mg of protein) and on MDA content (nmol/mg of protein) in leaves and roots of *D. stramonium* seedlings compared to the control group.

t		24 h	72 h	120 h
Leaves				
Catalase	Control	72.04 ± 6.75^{a}	80.45 ± 1.33^{a}	74.92 ± 5.55^{a}
	0.1 %	91.43 ± 14.66^{a}	73.26 ± 13.01^{a}	65.66 ± 2.08^{a}
	0.2 %	90.52 ± 5.30^{a}	71.86 ± 10.27^{a}	38.57 ± 4.69^{b}
Superoxide dismutase	Control	27.07 ± 1.02^{a}	32.51 ± 0.15^{b}	31.96 ± 0.43^{b}
	0.1 %	35.36 ± 0.92^{e}	20.70 ± 0.17^{d}	29.33 ± 0.21^{c}
	0.2 %	25.84 ± 0.23^{a}	25.97 ± 0.09^{a}	38.20 ± 0.56^{f}
Guaiacol peroxidase	Control	(0.76 ± 0.03)·10$^{3\ a}$	(0.80 ± 0.09)·10$^{3\ a}$	(1.42 ± 0.09)·10$^{3\ c,d}$
	0.1 %	(0.82 ± 0.01)·10$^{3\ a}$	(1.12 ± 0.07)·10$^{3\ b}$	(1.25 ± 0.06)·10$^{3\ c}$
	0.2 %	(0.97 ± 0.05)·10$^{3\ a,b}$	(1.50 ± 0.11)·10$^{3\ d}$	(1.65 ± 0.04)·10$^{3\ d}$
Pyrogallol peroxidase	Control	(0.64 ± 0.06)·10$^{3\ a}$	(0.91 ± 0.077.86)·10$^{3\ a,c}$	(0.67 ± 0.05)·10$^{3\ a}$
	0.1 %	(0.79 ± 0.05)·10$^{3\ a,b,c}$	(1.02 ± 0.04)·10$^{3\ c}$	(1.32 ± 0.09)·10$^{3\ d}$
	0.2 %	(0.69 ± 0.08)·10$^{3\ a,b}$	(1.41 ± 0.13)·10$^{3\ d}$	(1.04 ± 0.03)·10$^{3\ c}$
MDA content	Control	6.90 ± 1.57^{a,b}	11.24 ± 2.08^{a,b}	6.33 ± 0.09^{b}
	0.1 %	9.88 ± 1.13^{a,b}	9.07 ± 3.00^{a,b}	6.32 ± 0.38^{b}
	0.2 %	12.15 ± 2.44^{a}	7.17 ± 0.61^{a,b}	7.09 ± 0.14^{a,b}
Roots				
Catalase	Control	23.64 ± 2.58^{a,b}	21.93 ± 1.81^{a}	17.86 ± 2.83^{a}
	0.1 %	34.43 ± 2.22^{a,b}	32.89 ± 3.46^{a,b}	57.38 ± 10.72^{b}
	0.2 %	29.29 ± 3.71^{a,b}	46.77 ± 8.63^{a,b}	80.27 ± 27.58^{c}
Superoxide dismutase	Control	68.79 ± 3.22^{a}	57.16 ± 4.09^{a,b}	96.37 ± 0.83^{c}
	0.1 %	103.57 ± 20.23^{c}	63.75 ± 7.06^{a,b}	99.96 ± 5.42^{c}
	0.2 %	39.77 ± 8.00^{b}	54.06 ± 10.58^{a,b}	118.97± 3.66^{c}
Guaiacol peroxidase	Control	(4.58 ± 0.25)·10$^{3\ a,b}$	(5.60 ± 0.27)·10$^{3\ b,c}$	(6.76 ± 0.27)·10$^{3\ e}$
	0.1 %	(3.87 ± 0.46)·10$^{3\ a}$	(4.85 ± 0.10)·10$^{3\ b}$	(10.00 ± 0.22)·10$^{3\ f}$
	0.2 %	(2.93 ± 0.13)·10$^{3\ d}$	(5.80 ± 0.26)·10$^{3\ c}$	(3.92 ± 0.47)·10$^{3\ a}$
Pyrogallol peroxidase	Control	(4.36 ± 0.34)·10$^{3\ a}$	(5.61 ± 0.28)·10$^{3\ c}$	(3.77 ± 0.24)·10$^{3\ a,b}$
	0.1 %	(4.42 ± 0.19)·10$^{3\ a}$	(3.76 ± 0.64)·10$^{3\ a,b}$	(3.23 ± 0.09)·10$^{3\ b}$
	0.2 %	(3.63± 0.16)·10$^{3\ a,b}$	(4.28 ± 0.21)·10$^{3\ a}$	(3.25 ± 0.26)·10$^{3\ b}$
MDA content	Control	8.22 ± 1.09^{a,b}	8.15 ± 0.03^{a,b}	7.36 ± 0.27^{a}
	0.1 %	5.98 ± 0.17^{a}	21.08 ± 7.05^{c}	12.13 ± 0.64^{a,b,c}
	0.2 %	23.99 ± 4.83^{d}	18.34 ± 3.89^{b}	12.34 ± 0.11^{a,b,c}

The data are mean values ± standard error. $^{a\text{-}f}$values without the same superscripts within each column differ significantly (P<0.05).

nism of allelopathy (Elisante & Ndakidemi 2014). It is known that root exudates are one of the main sources of allelochemicals released into the soil (Gatti *et al*. 2010). In line with this, it is very important to remove them from crops. The aim of this study was to investigate the impact of winter savory (*S. montana*) aqueous extract in natural weed management. The effect of *S. montana* aqueous extract on the lipid peroxidation process and activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase, and pyrogallol peroxidase in leaves and roots of Jimson weed (*D. stramonium*) seedlings is examined in the study.

Plant material. The wild aromatic plant *S. montana* was collected around the city of Podgorica in Montenegro (42°32'23.21'' N, 19°20'02.17'' E) at an elevation of 123 m a.s.l. in June of 2012 (voucher No. 2-1544). *Datura stramonium* seeds were collected in Futog (Krndelja; UTM: 34T DR 2 01) in June of 2012 (Voucher No. 2-1486). Voucher specimens of both species are deposited in the herbarium of the Department of Biology and Ecology (BUNS), Faculty of Natural Sciences, University of Novi Sad (Holmgren & Holmgren 2003).

Preparation of aqueous extract. Air–dried plant material (above-ground part) from *S. montana* was ground into powder. The powder (10 g) was extracted in 100 mL of boiling distilled water (10% w/v).

Seedling growth. The experiment was performed at the Laboratory of Biochemistry, Faculty of Agriculture, Novi Sad, and conducted under controlled conditions (28°C, 60% relative humidity, 18-h photoperiod, and light intensity of 10,000 lx). *Datura stramonium* seeds were surface-sterilised with 3% H_2O_2 (v/v) and washed with sterilised deionised water. These seeds were placed in plastic pots containing sterile sand and maintained under dark conditions. Thirty-day-old seedlings were transplanted in plastic pots containing 700 mL of Hoagland's solution [10% $MgSO_4 \times 7H_2O$, 10% $Ca(NO_3)_2 \times 4\ H_2O$, 10% KH_2PO_4, 10% KNO_3, microelements, 7.5% Fe-EDTA] and 7 (0.1 %) or 14 (0.2 %) mL of *S. montana* aqueous extract, while pots of the control contained the same volume of nutrient solution without addition of *S. montana* aqueous extract. Seedlings were harvested for determining the investigated biochemical parameters 24, 72, and 120 h after the treatments with *S. montana* aqueous extract.

Biochemical assays. For determination of oxidative stress parameters, 2 g of fresh plant material (leaves and roots of *D. stramonium* plants, separately) was crushed and homogenised in 10 mL of phosphate buffer (0.1 M, pH 7.0). Homogenates were centrifuged for 20 min at 10.000 x g and filtered. The supernatants were used for biochemical assays. Lipid peroxidation was measured at 532 nm using the thiobarbituric acid (TBA) test (Mandal *et al.* 2008). The total amount of TBA–reactive substances was given in nmol of malondialdehyde (MDA) equivalents per mg of proteins. Catalase (CAT) (EC 1.11.1.6) activity was determined according to Sathya & Bjorn (2010). The decomposition of H_2O_2 was followed as the decrease in absorbance at 240 nm. Activity of the enzyme was expressed in U mg^{-1} of proteins. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed according to a slightly modified version of the method of Mandal *et al.* (2008) by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) chloride. One unit of SOD activity was defined as the amount of the enzyme required to inhibit reduction of NBT by 50%. Peroxidase (POD) (EC 1.11.1.7) activity was measured using guaiacol (guaiacol peroxidase) and pyrogallol (pyrogallol peroxidase) as substrates according to Morkunas & Gmerek (2007). Activity of the enzyme was expressed in U mg^{-1} of proteins.

Statistical analyses. All measurements were performed in triplicates. Values of the biochemical parameters were expressed as means ± standard error of the mean and tested by ANOVA, followed by comparison of the means by Duncan's multiple range test ($P<0.05$). Data were analysed using STATISTICA for Windows version 11.0.

Allelopathic interactions and natural substances present in plant extracts are good candidates to be developed as bioherbicides. In the present study, a significant decrease of catalase activity was detected in the leaves of Jimson weed seedlings treated with a 0.2% concentration of *S. montana* aqueous extract 120 h after the treatment. A significant decrease of superoxide dismutase activity and significant increase of guaiacol and pyrogallol peroxidase activity were observed in the variants of treatments with both tested concentrations 72 h after the treatment (Table 1). In the roots of Jimson weed seedlings, a significant increase of catalase activity was recorded 120 h after treatment with 0.2% *S. montana* aqueous extract. Statistically significant increases in MDA accumulation were recorded in roots of Jimson weed 24 h after treatment with a 0.2% concentration and 72 h after treatment with a 0.1% concentration of *S. montana* aqueous extract. The results showed that the tested doses did not induce lipid peroxidation in the leaves of Jimson weed seedlings. On the other hand, both concentrations induced higher accumulation of MDA, an end-product of the lipid peroxidation process, in the roots of Jimson weed seedlings during the first 72 h (Table 1). These results show that the roots were more affected than the leaves, the reason for this probably lying in fact that the roots were directly exposed to *S. montana* aqueous extract. Other studies have also shown the allelopathic potential of *S. montana*. Angelini *et al.* (2003) found that *S. montana* essential oil blocked germination of three weed species,

viz., lambsquarters (*Chenopodium album* L.), little hogweed (*Portulaca oleracea* L.), and barnyard grass [*Echinochloa crus-galli* (L.) Beauv.]. Furthermore, the aqueous extracts of other *Satureja* species were shown to have inhibitory effects on the percentage of weed seed germination (Gholami *et al.* 2011).

Our results showed that roots of Jimson weed were more affected by *S. montana* aqueous extract than leaves. Both tested concentrations of *S. montana* aqueous extract induced lipid peroxidation in Jimson weed roots 24 h and 72 h after the treatment. Sensitivity of *D. stramonium* to *S. montana* aqueous extract indicates that natural substances present in plant extracts are good candidates to be developed as bioherbicides.

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Botanica SERBICA

REZIME

Uticaj vodenog ekstrakta *Satureja montana* L. na antioksidativna svojstva tatule (*Datura stramonium* L.)

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Zbog sve veće otpornost korovskih vrsta prema herbicidima, kao i problema koji se javljaju nakon upotrebe sintetičkih herbicida, ulažu se veliki napori u izradi alternativnih strategija upravljanja korovom. Ovo istraživanje je sprovedeno kako bi se ispitao uticaj vodenog ekstrakta rtanjskog čaja (*Satureja montana*) kao prirodnog herbicida u upravljanju korovom. Ispitan je uticaj dve koncentracije (0.1 i 0.2%) vodenog ekstrakta *S. montana* na aktivnost antioksidativnih enzima i proces lipidne peroksidacije u sadnicama tatule (*Datura stramonium*). Dobijeni rezultati su pokazali da je povećanje intenziteta lipidne peroksidacije zabeleženo u korenu tatule 72 časa nakon tretmana.

Ključne reči: alelopatija, *Datura stramonium*, *Satureja montana*