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Oxidative stress in bromus (*Bromus mollis* L.) seedlings treated with clary sage (*Salvia sclarea* L.) aqueous extract

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***Abstract*:**

Šućur, J., Prvulović, D., Anačkov, G., Malenčić, Đ.: ***Oxidative stress in bromus (Bromus mollis L.) seedlings treated with clary sage (Salvia sclarea L.) aqueous extract. Biologica Nyssana, 7 (2), December 2016: 141-144.***

Extensive use of synthetic pesticides has negative effects on the environment and on human and animal health. Knowledge on allelopathic interactions could provide effective tools for a better exploitation of natural resources in the management of weeds without using herbicides. One of highly resistant weed species is bromus. The effects of two concentrations (0.1% and 0.2%) of *Salvia sclarea* L. aqueous extract on the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) in leaves and roots of bromus (*Bromus mollis* L.) seedlings, were examined. Our results showed that both concentrations of the extract used (0.1% and 0.2%) stimulated the significant increase of the superoxide dismutase activity in leaves and roots of bromus 72 hours and 120 hours after the treatment. The significant increase of the catalase activity was recorded in roots of bromus 72 h after the treatment. Two tested extract concentrations affected activity of the antioxidant enzymes in the same way, but the higher activity was observed in the roots treated with higher concentration (0.2%). The increase of the activities of antioxidant enzymes, in response to stress induced by *S. sclarea* aqueous extract, indicate that the plant extract possesses allelopathic activity on treated plant.

Key words: Allelopathy, Biopesticides, *Bromus mollis* L., *Salvia sclarea* L.

***Apstrakt*:**

Šućur, J., Prvulović, D., Anačkov, G., Malenčić, Đ.: ***Oksidativni stres u sadnicama klasače (Bromus mollis L.) tretiranim vodenim ekstraktom muskatne žalfije (Salvia sclarea L.). Biologica Nyssana, 7 (2), Decembar 2016: 141-144.***

Upotreba sintetičkih pesticida ima negativan efekat na životnu sredinu, kao i zdravlje ljudi i životinja. Poznavanje alelopatskih interakcija može da obezbedi efikasniji način eksploatacije prirodnih resursa u suzbijanju korova bez upotrebe sintetičkih herbicida. Jedna od otpornijih vrsta korova je korov klasača. U ovom radu je ispitan uticaj dve koncentracije (0,1% i 0,2%) vodenog ekstrakta *S. sclarea* na aktivnost antioksidantnih enzima superoksid-dismutaze (SOD) i katalaze (CAT) u listu i korenu klasače (*Bromus mollis* L.). Dobijeni rezultati su pokazali statistički značajno povećanje aktivnosti enzima superoksid-dismutaze u

listu i korenu klasače, 72 i 120 časova nakon tretmana za obe primenjene koncentracije ekstrakata (0,1% i 0,2%). Statistički značajno povećanje aktivnosti katalaze uočeno je u korenu klasače 72 časa nakon tretmana. Obe ispitane koncentracije vodenih ekstrakata uticale su na aktivnost antioksidantnih enzima, pri čemu je veća aktivnost uočena u korenu klasače tretirane sa višom koncentracijom (0,2%). Povećanje aktivnosti antioksidantnih enzima, kao odgovor na stres prouzrokovan vodenim ekstraktom *S. sclarea*, pokazuje da ekstrakt poseduje alelopatski uticaj na tretiranu biljku.

Ključne reči: Alelopatija, *Glycine max* (L.) Merr., *Satureja montana* L.

Introduction

The flowering plant family of Lamiaceae is very important since its highly diverse species are potent source of secondary metabolites (Fakoorziba et al., 2014). Numerous species of the genus *Salvia*, belonging to Lamiaceae, have been used in traditional medicine (Nasermoadeli & Rowshan, 2013). *Salvia sclarea* L., known as clary sage, a plant native to southern Europe, is one of the most important aromatic plants cultivated world-wide as a source of essential oils and many other compounds derived from different parts of the plant (Hudaib et al., 2001, Nasermoadeli & Rowshan, 2013).

Plant-derived chemical compounds are also involved in communication between plants (Weir et al., 2004). Allelopathy is an interference mechanism in which plants release chemical substances, called allelochemicals, from leaves, stems, roots, flowers and seeds which inhibit or stimulate plant growth (Gella et al., 2013). Accordingly, allelopathy is a natural and an environment-friendly technique which may prove to be also a unique tool for weed control (Sharma & Satsangi, 2013). Allelochemicals are highly attractive as new classes of herbicides due to a variety of advantages. Most of them are water-soluble which makes them easier to apply and their chemical structures are more environmentally friendly than synthetic ones (Soltys et al., 2013).

The aim of this study was to examine the effect of two concentrations (0.1% and 0.2%) of *Salvia sclarea* L. aqueous extract on the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in leaves and roots of bromus (*Bromus mollis* L.) seedlings and to explore the biopesticidal potential of this species in weed control.

Material and methods

The wild, aromatic plant, *S. sclarea* L., was collected on Mt Rujan, in the south of Serbia (N 42º22′40.44″, E 21º53′09.23″, 494 m.a.s.l.) in July of 2012. Voucher specimens of collected plant was confirmed and deposited at the Herbarium of the Department of Biology, Faculty of Natural Sciences, University of Novi Sad (BUNS 2-1545). The aqueous extract of *S. sclarea* was prepared with air-dried plant material (10 g) in boiling distilled water (100 ml). After 24 h, the extract was filtered through Whatman No.4 filter paper and kept at 4 °C in the fridge until application.

The experiment was performed at the Laboratory of Biochemistry, Faculty of Agriculture, Novi Sad and conducted under controlled conditions (28 ºC, 60% relative humidity, a photoperiod of 18 h, and a light intensity of 10.000 lx). The bromus (*Bromus mollis* L.) seeds were grown in plastic pots containing sterile sand. After 30 days, the plants were transplanted in plastic pots containing 700 ml of Hoagland's solution and 7 and 14 ml of *S. sclarea* aqueous extract, while pots of control contained just the same volume of Hoagland's solution (10% $MgSO_4$ x $7H_2O$, 10% $Ca(NO_3)_2$ x 4 H_2O, 10% KH_2PO_4, 10% KNO_3, microelements, 7.5% Fe-EDTA). Plants were harvested for further biochemical analyzes 24, 72 and 120 h after the treatments. Fresh leaves and roots of bromus plants (2 g each) were homogenized in 10 ml of phosphate buffer (0.1 M, pH 7.0). Homogenates were centrifuged for 20 min at 10.000 x *g* (Boeco, Germany) and filtered. The supernatants were used for determining the investigated biochemical parameters (activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT)). Biochemical analyses were carried out spectrophotometrically using an UV/VIS spectrophotometer model Thermo Scientific Evolution 220 (USA).

The catalase (CAT) (EC 1.11.1.6) activity was determined according to Sathya & Bjorn, (2010). The decomposition of H_2O_2 was followed as a decrease in absorbance at 240 nm. The enzyme extract was added to the assay mixture containing 1 mL of 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2 (Centrohem, Serbia). The activity of the enzyme is expressed as U per 1 g of protein (U mg^{-1} protein). Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed according to a slightly modified method of Mandal et al. (2008) by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) chloride. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM L-methionine (Sigma (St.

Louis, Mo.)), 75 μM NBT (Sigma (St. Louis, Mo.)), 0.1 mM EDTA, 2 μM riboflavin (Sigma (St. Louis, Mo.)) and 0.02 mL of the enzyme extract. It was kept under a fluorescent lamp for 30 min, and then the absorbance was read at 560 nm. One unit of the SOD activity is defined as the amount of enzyme required to inhibit the reduction of NBT by 50%. The activity of the enzyme is expressed as U per 1 mg of protein (U mg^{-1} protein).

Values of the biochemical parameters were expressed as means ± standard error (*SE*) of determinations made in triplicates and tested by ANOVA followed by comparison of the means by the Duncan multiple range test ($P < 0.05$). Data were analyzed using Statistica for Windows, version 11.0. The results are presented as histograms (values marked with different letter differ significantly at $P < 0.05$).

Results and discussion

In the leaves of bromus seedlings, the significant increase in activity of CAT was detected in the treatment with lower concentrations of the *S. sclarea* aqueous extract 120 h after the treatment (**Fig. 1**). The highest activity of the SOD was observed in plants 120 h after the treatment with 0.2% *S. sclarea* aqueous extract (**Fig. 2**). In the roots of bromus, the significant increase in CAT activity was recorded 72 and 120 h after the treatment (**Fig. 1**). The activity of SOD was significantly increased 72 and 120 h after the treatments with *S. sclarea* aqueous extract (**Fig. 2**).

Numerous growth inhibitors identified in some plants are responsible for their allelopathic properties and may be a useful source for the future development of bioherbicides (Xuan et al., 2005). In the research of Sharma & Satsangi (2013)

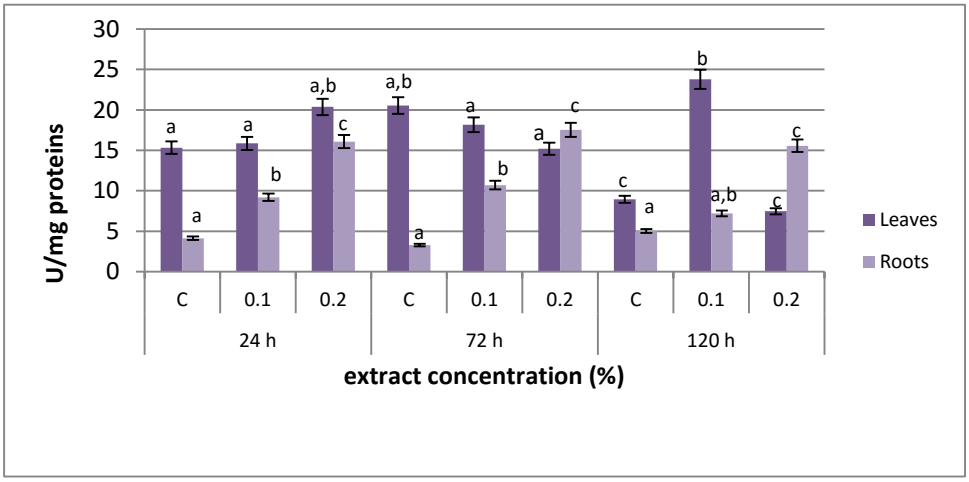

Fig. 1. Activity of CAT in leaves and roots of bromus seedlings 24, 72 and 120 h after the treatment with different concentrations (%) of *S. sclarea* aqueous extracts (v/v) and in control (C).

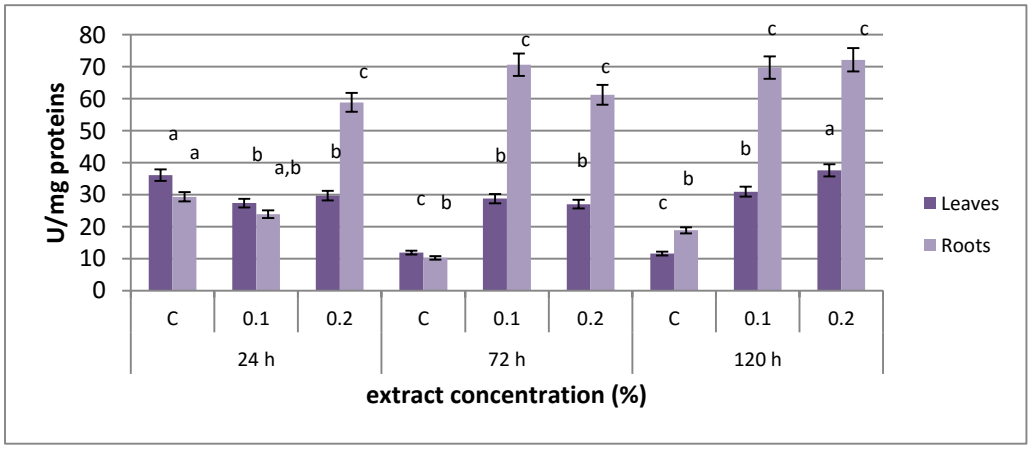

Fig. 2. Activity of SOD in leaves and roots of bromus seedlings 24, 72 and 120 h after the treatment with different concentrations (%) of *S. sclarea* aqueous extracts (v/v) and in control (C)

the aqueous leaf extract of the sunflower (*Helianthus annuus* L.) showed an inhibitory effect on germination and growth of *Parthenium hysterophorus.* This negative effect came from allelochemicals present in the aqueous extract of the sunflower. Hussain & Reigosa (2011) reported that allelochemical cinnamic acid, widespread phenolic acid, was phytotoxic to C_3 perennial plant species (*Dactylis glomerata*, *Lolium perenne* and *Rumex acetosa*). The toxicity of the extracts varied with the concentration and soaking periods. The extract with higher concentration and longer soaking periods were more inhibitory then lower (Husna et al., 2016). Further, lower concentrations of plant's aqueous extracts could be more inhibitory in sterile soil than in nonsterile soil (Barnes & Putnam, 1986).

Our results showed that both concentrations of *S. sclarea* extract used (0.1% and 0.2%), stimulated the significant increase of the superoxide dismutase activity in leaves and roots of bromus 72 and 120 h after the treatment. The two tested extract concentrations affected the activity of the catalase in the bromus roots in the same way, but a higher activity was observed in the treatment with the higher concentration of the *S. sclarea* aqueous extracts (0.2%). The increases in the activities of antioxidant enzymes probably occur in response to stress induced by *S. sclarea* aqueous extract.

Conclusion

In conclusion, our results showed that *S. sclarea* aqueous extract stimulates an increase of the catalase activity in the roots of bromus seedlings while both tested concentrations caused a significant increase of the superoxide dismutase activity in bromus leaves and roots. The results indicate that the plant extract possesses allelopathic activity and increases in activity of antioxidant enzymes probably occur in response to stress.

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