

Original Article

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The effect of *Satureja montana* L. aqueous extract on soybean seedlings

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Abstract:

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The aim of this study was to examine the impact of *Satureja montana* L. aqueous extract on soybean antioxidant properties so as to assess its possible side effects when applied as bioherbicide in soybean organic production. The effects of two concentrations (0.1% and 0.2%) of *S. montana* aqueous extract on the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in leaves and roots of soybean (*Glycine max* L.) seedlings were examined 24, 72 and 120 h after the treatment. Our results showed that the significant increase in the catalase activity was recorded in roots of soybean treated with both concentrations of the extract used. On the other hand, both concentrations of *S. montana* aqueous extract stimulated the significant increase of the superoxide dismutase activity in leaves and roots of soybean. Higher activity of the antioxidant enzymes in the roots of soybean compared with activity of the antioxidant enzymes in leaves showed that roots were more affected than leaves.

Key words: Allelopathy, *Glycine max* (L.) Merr., *Satureja montana* L.

Apstrakt:

Šučur, J., Prvulović, D., Anačkov, G., Malenčić, Đ.: *Efekat vodenog ekstrakta Satureja montana* L. na sadnice soje. *Biologica Nyssana*, 7 (2), Decembar 2016: 125-129.

Cilj ovog rada je ispitivanje uticaja vodenog ekstrakta *Satureja montana* L. na parametre oksidativnog stresa u sadnicama soje kako bi se procenile eventualne nuspojave nakon primene ovog ekstrakta kao bioherbicida u organskoj proizvodnji soje. Ispitan je uticaj dve koncentracije (0,1% i 0,2%) vodenog ekstrakta *S. montana* na aktivnost antioksidantnih enzima superoksid-dismutaze (SOD) i katalaze (CAT) u listu i korenu sadnica soje (*Glycine max* L.) 24, 72 i 120 časova nakon tretmana. Dobijeni rezultati su pokazali statistički značajno povećanje aktivnosti enzima katalaze u korenu soje, kao i statistički značajno povećanje aktivnosti superoksid-dismutaze u listu i korenu soje, u tretmanima za obe primenjene koncentracije ekstrakata. Veća aktivnost antioksidantnih enzima u korenu soje, u poređenju sa aktivnošću u listu, ukazuje na veću senzitivnost korena od lista prema primenjenom ekstraktu.

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

Introduction

Satureja montana L. (winter savory or mountain savory), belonging to the Lamiaceae family, native to the Mediterranean regions, is a well-known aromatic plant which contains various biologically active constituents such as essential oils, triterpenes, flavonoids and rosmarinic acid (Hassanein et al., 2014; Dudaš et al., 2013). It is found throughout Europe, Russia and Turkey, growing in sunny, stony and rocky regions (Trifan et al., 2015). Owing to the active components and pleasant aroma, winter savory is often used as a medicinal plant and a spice (Dudaš et al., 2013; Trifan et al., 2015).

The plant releases many bioactive chemicals from its various parts (leaves, stems, roots) into its surrounding environment, which are called allelochemicals (Mondal et al., 2015). Allelopathy is an interference mechanism by which plants release allelochemicals which affect the growth of other plants (Bajalan et al., 2013). Allelopathic interaction is either positive or negative (Mondal et al., 2015). Released allelochemicals become stressful only when they are toxic or when they affect the growth and development of surrounding plants (Cruz-Ortega et al., 2007). Increasing attention has been given to the role and potential of allelopathy as a management strategy for crop protection against weeds and other pests (Bajalan et al., 2013).

The aim of this study was to examine the effect of aqueous extract of *S. montana* on soybean (*Glycine max* (L.) Merr.) antioxidant properties so as to assess its possible side effects when applied as bioherbicide in soybean organic production.

Material and methods

The aromatic plant, *S. montana*, was collected at localities near the Adriatic coast in Montenegro, in June, 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 2-1544). The air-dried plant material was ground into powder. The powdery material (10 g) was extracted with 100 mL distilled water. After 24 h, the extract was filtered through filter paper and kept at 4 °C until application.

The experiment was performed at the Laboratory of Biochemistry, Faculty of Agriculture, Novi Sad and conducted under controlled conditions (temperature 28 °C, 60% relative humidity, 18 h photoperiod, a light intensity of 10.000 lx). Soybean seedlings (*G. max*) cv. Viktorija were grown for 30 days in plastic pots (500 ml) containing sterile sand, after which they were transferred on plastic pots (700 ml) containing the Hoagland's solution and 7 and 14

ml of *S. montana* aqueous extract, while 7 and 14 ml of the Hoagland's solution were added in pots of control. Seedlings were harvested for determining the investigated biochemical parameters 24, 72 and 120 h after the treatments. Fresh leaves and roots of soybean 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 and filtered. The supernatants were used to test activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT).

The catalase (CAT) (EC 1.11.1.6) activity was determined according to Sathya & Bjorn (2010). The decomposition of H₂O₂ was followed as a decrease in absorbance at 240 nm. The enzyme extract was added to the assay mixture containing 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H₂O₂. 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, 75 μM NBT, 0.1 mM EDTA, 2 μM riboflavin 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.

Results and discussion

Allelochemicals can cause oxidative stress in target plants and therefore activate the antioxidant mechanism (Cruz-Ortega et al., 2007). Accordingly, the response of plants to damaging adverse circumstances is closely related to their enzyme activity (Sunmonu & Van Staden, 2014). Enzymes, such as superoxide dismutases (SOD), catalases (CAT) and peroxidases, play important roles in protecting cells against reactive oxygen species (Kuthan et al., 1986). Therefore, the activity of those enzymes can be used as indicators of oxidative stress in plants (An et al., 2005).

Our results showed that a significant increase in the catalase activity was recorded in roots of soybean seedlings treated with both concentrations of the extract used 72 h after the treatment (Fig. 1). On the other hand, both concentrations of *S. montana* aqueous extract stimulated a significant increase of the superoxide dismutase activity in roots of soybean seedlings 72 and 120 h after the treatment (Fig. 2).

The increases in activity of antioxidant enzymes probably occur in response to stress. A higher activity of the antioxidant enzymes in the roots of soybean seedlings compared with the activity of the antioxidant enzymes in leaves showed that roots were more affected than leaves. The activity of examined antioxidant enzymes showed a downward

trend with the duration of the experiment, thus the highest activity of the catalase and superoxide dismutase was observed after 72 h. This could point to the fact that scavenging effects of superoxide dismutase and catalase could prevent an oxidative burst and the induction of lipid peroxidation process.

Conclusion

In conclusion, our results showed that *S. montana* aqueous extract did not stimulate an increase of the catalase and superoxide dismutase activity in the leaves of soybean seedlings, while both tested concentrations caused a significant increase in

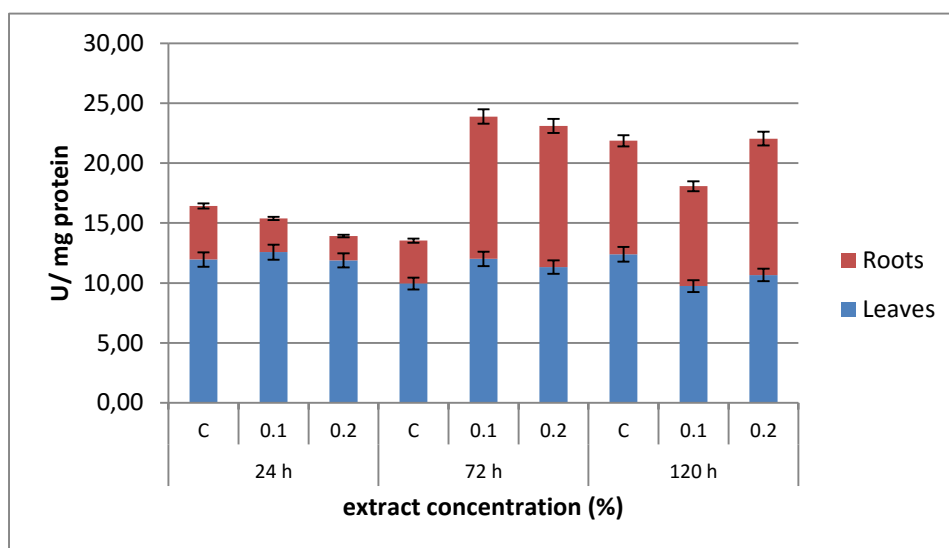


Fig. 1. Activity of catalase in leaves and roots of soybean seedlings 24, 72 and 120 h after treatment with different concentrations (%) of *S. montana* aqueous extracts (v/v) and in control (C).

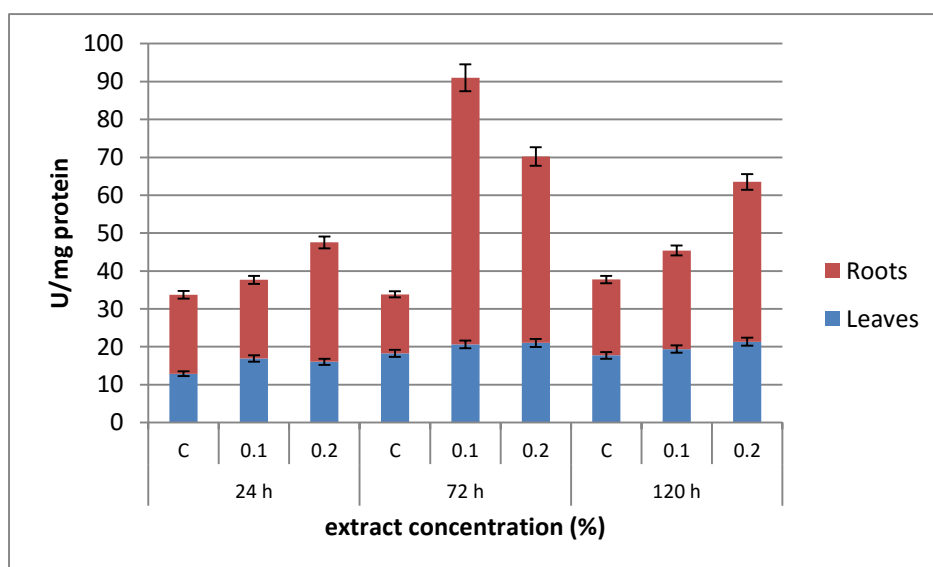


Fig. 2. Activity of superoxide dismutase in leaves and roots of soybean seedlings 24, 72 and 120 h after treatment with different concentrations (%) of *S. montana* aqueous extracts (v/v) and in control (C).

soybean roots. This may be attributed to the permeability of allelochemicals to root tissues arising from direct contact with the phytotoxic compounds present in the extract.

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References

- Assyov, B., Denchev, C. 2015: *Verbascum davidoffii* Murb. In: Biserkov, V. et al. (eds.), Digital edition of Red Data Book of Republic of Bulgaria. BAS & MOEW, Sofia.
<http://e-ecodb.bas.bg/rdb/en/vol1/Verdavid.html>.
- Biserkov, V. et al. (eds.) 2015: Digital edition of Red Data Book of Republic of Bulgaria. BAS & MOEW, Sofia. <http://e-ecodb.bas.bg/rdb/en/>.
- Camacho, F.J., Liston, A. 2001: Population structure and genetic diversity of *Botrychium pumicola* (Ophioglossaceae) based on inter-simple sequence repeats (ISSR). *American Journal of Botany*, 88 (6): 1065-1070.
- Doyle, J.J., Doyle, J.L. 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19: 11-15.
- Fang, D.Q., Roose, M.L. 1997: Identification of closely related citrus cultivars with inter-simple sequence repeat genetics. *Theoretical and Applied Genetics*, 95 (3): 408-417.
- Firat, M. 2015: *Verbascum kurdistanicum* (Scrophulariaceae), a new species from Hakkari, Turkey. *PhytoKeys*, 52: 89-94.
- Gonzalo-Turpin H., Hazard, L. 2009: Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, 97 (4): 742-751.
- Hamrick, J.L., Godt, M.J.W. 1996: Conservation genetics of endemic plant species. In: Avise, J.C., Hamrick, J.L. (eds.), Conservation genetics: case histories from nature. Chapman and Hall, New York, pp. 281-304.
- Helenurm, K. 2001: High levels of genetic polymorphism in the insular endemic herb *Jepsonia malvifolia*. *Journal of Heredity*, 92 (5): 427-432.
- Huang, Y., Zhang, C.Q., Li, D.Z. 2009: Low genetic diversity and high genetic differentiation in the critically endangered *Omphalogramma souliei* (Primulaceae): implications for its conservation. *Journal of Systematics and Evolution*, 47 (2): 103-109.
- Idrees, M., Irshad, M. 2014: Molecular markers in plants for analysis of genetic diversity: a review. *European Academic Research*, 2 (1): 1513-1540.
- Jia, J., Zeng, L., Gong, X. 2016: High genetic diversity and population differentiation in the critically endangered plant species *Trailliaedoxa gracilis* (Rubiaceae). *Plant Molecular Biology Reporter*, 34 (1): 327-338.
- Li, Y.Y., Guan, S.M., Yang S.Z., Luo, Y., Chen, X.Y. 2012: Genetic decline and inbreeding depression in an extremely rare tree. *Conservation Genetics*, 13 (2): 343-347.
- Nyblom, H. 2004: Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, 13 (5): 1143-1155.
- Peakall, R., Smouse, P.E. 2012: GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics*, 28 (19): 2537-2539.
- Petrova, G., Dzhambazova, T., Moyankova, D., Georgieva, D., Michova, A., Djilianov, D., Moeller, M. 2014: Morphological variation, genetic diversity and genome size of critically endangered *Haberlea* (Gesneriaceae) populations in Bulgaria do not support the recognition of two different species. *Plant Systematics and Evolution*, 300 (1): 29-41.
- Rao, V.R., Hodgkin, T. 2002: Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue Organ Culture*, 68 (1): 1-19.
- Stefanova-Gateva, B. 1995: *Verbascum* L. In: Kožuharov, S. (ed.), Flora Reipublicae Popularis Bulgaricae 10: 26-100, Acad. "Prof. M. Drinov", Serdicae, Sofia, Bulgaria (in Bulgarian).
- Turchetto, C., Segatto, A.L.A., Mäder, G., Rodrigues, D.M., Bonatto, S.L., Freitas, L.B. 2016: High levels of genetic diversity and population structure in an endemic and rare species: implications for conservation. *AoB PLANTS* 8: plw002; doi:10.1093/aobpla/plw002.
- Walther, G.-R., Beißner, S., Burga, C.A. 2005: Trends in the upward shift of alpine plants. *Journal of Vegetation Science*, 16 (5): 541-548.
- Willi, Y., Van Buskirk, J., Hoffmann, A.A. 2006: Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution and Systematics*, 37: 433-458.
- Wolfe, A.D., Liston, A. 1998: Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis, P.S., Soltis, D.E., Doyle, J.J. (eds.), Molecular systematics of plants: DNA sequencing. Kluwer, New York, pp. 43-86
- Xue, D.-W., Ge, X.-J., Hao, G., Zhang, C.-Q. 2004: High genetic diversity in a rare, narrow endemic

- primrose species: *Primula interjacens* by ISSR analysis. *Acta Botanica Sinica*, 46 (10): 1163-1169.
- Zawko, G., Krauss, S.L., Dixon, K.W., Sivasithamparam, K. 2001: Conservation genetics of the rare and endangered *Leucopogon obtectus* (Ericaceae). *Molecular Ecology*, 10 (10): 2389-2396.
- Zhu, Y., Geng, Y., Tersing, T., Liu, N., Wang, Q., Zhong, Y. 2009: High genetic differentiation and low genetic diversity in *Incarvillea younghusbandii*, an endemic plant of Qinghai-Tibetan Plateau, revealed by AFLP markers. *Biochemical Systematics and Ecology*, 37 (5): 589-596.
- Zietkiewicz, E., Rafalski, A., Labuda, D. 1994: Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20 (2): 176-183.

