BIOLOGICA NYSSANA
4 (1-2) • December 2013: 71-74
11th SFSES • 13-16 June 2013, Vlasina lake

Original Article

Oxidative stress in soybean seedlings treated with *Thymus serpyllum* aqueous extract

Jovana Šućur, Milan Popović, Dejan Prvulović, Đorđe Malenčić, Biljana Kiprovski

Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

** E-mail: bkiprovski@polj.uns.ac.rs*

***Abstract*:**
Šućur, J., Popović, M., Prvulović, D., Malenčić, Đ., Kiprovski, B.: *Oxidative stress in soybean seedlings treated with Thymus serpyllum aqueous extract*. *Biologica Nyssana, 4 (1-2), December 2013: 71-74.*

The effect of different concentrations (0.05, 0.1 and 0.2%) of *Thymus serpyllum* L. aqueous extracts on lipid peroxidation process (LP), as well as reduced glutathione content (GSH) in leaves and roots of soybean seedlings were examined 24 and 72 h after the treatment. Our results showed that only highest concentration of the extract used (0.2%) enhanced process of lipid peroxidation, while concentration of 0.1% stimulated GSH accumulation in soybean seedlings.

Key words: allelopathy, antioxidants, biopesticides, *Glycine max* L., *Thymus serpyllum* L.

Introduction

The genus *Thymus* L. (Lamiaceae) consists of about 215 species of herbaceous perennial and subshrubs. *Thymus* species are considered as medicinal plants due to their pharmacological and biological properties (Hussain et al., 2013). It is commonly used as tonic, herbal tea and flavouring agent. Thymus oils and extracts are widely used in pharmaceutical, cosmetic and perfume industry also for flavouring and bio-preservation of several food products (Verma et al., 2011). In the Mediterranean region, which can be described as the centre of this genus, annual aromatic herb, *Thymus serpyllum* L. inhabits cultivated and uncultivated lands including wastelands (Verma et al., 2011). Research on allelopathy of *T. serpyllum* on weeds shown that water extract of *T. serpyllum* promoted test plants' growth at low concentration but inhibited them at high, and inhibition became stronger as concentration increased (Zhang, 2009).

The use of allelochemicals as weed control agents, becomes widely investigated, however the impact of these bioherbicide on cultivated plants is less known. Since ROS (Reactive Oxygen Species)-generation resulting in oxidative damage has been proposed as one of the modes of action of allelochemicals, the aim of this study was to examine the impact of *Thymus serpyllum* L. aqueous extract on soybean antioxidant properties so as to assess its possible side effects when applied as bioherbicide in soybean organic production.

Material and methods

The wild, aromatic plant, *Thymus serpyllum* L., was collected in northern Serbia (Vojvodina province) in May of 2010. 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. Seeds of soybean (*Glycine max* L.) cv. Sava were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Serbia.

The aqueous extract of *T. serpyllum* was prepared with air-dried plant material (10 g) in boiling distilled water (100 ml). 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 25 °C, 60-70% relative humidity, 16 h photoperiod). Soybean seedlings were grown for 14 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 3.5, 7 and 14 ml of *T. serpyllum* aqueous extract, while 3.5, 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 and 48 h after the treatments.

Biochemical analyses were carried out spectrophotometrically using an UV/VIS spectrophotometer model 6105 (Jenway, UK). Lipid peroxidation (LP) was measured at 532 nm using the thiobarbituric acid (TBA) test. The total amount of TBARS (TBA-reactive substances) is given as nmol malondialdehyde (MDA) equivalents mg^{-1} proteins (Mandal et al., 2008). Reduced glutathione (GSH) was determined according to Sedlak and Lindsay (1968) and expressed as μmol GSH mg^{-1} proteins.

Values of the biochemical parameters were expressed as means ± standard error of determinations made in triplicates 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.

Results and discussion

The toxicity of many allelochemicals can largely be attributed to the formation of semiquinone radicals that donate electrons to molecular oxygen, forming reactive oxygen species (ROS), such as superoxide anions ($O_2^{\cdot-}$), and more reactive hydroxyl ($^{\cdot}OH$) or hydroperoxyl ($HO_2^{\cdot}$) radicals (Hammondkosak & Jones, 1996). This plant-plant allelopathic interaction generates a cascade of signaling events-including Ca^{2+} influx and proton efflux that actives the NADPH-oxidase complex (generating $^{\cdot}O_2^{-}$) and pH-sensible cell wall peroxidases (producing H_2O_2), which produces an oxidative burst (Dos Santos et al., 2008). Enhancement of ROS production during the oxidative burst is one of the earliest reactions elicited in response to various abiotic and biotic stimuli. Some allelochemicals rapidly depolarize the cell membrane, increasing membrane permeability, inducing lipid peroxidation and causing a generalized cellular disruption that ultimately leads to cell death (Devi & Prasad, 1996; Zeng et al., 2001; Yu et al., 2003). The peroxidation of unsaturated lipids of biological membranes is the most prominent symptom of oxidative stress in animals and plants (Mandal et al., 2008). Malondialdehyde (MDA) content, an end-product of lipid peroxidation process, is used as oxidant bio-marker. It is suggested that different concentrations of allelochemicals induce production of MDA, however due to their antagonistic and synergistic effect MDA content is not always in reciprocity with these chemicals (Ding et al., 2007; Haddadchi & Gerivani, 2009).

Our results showed that 24 h after the treatment with *T. serpyllum* aqueous extracts no significant difference in the LP intensity in soybean leaves and roots between plants form control and treatments were recorded. The significant increase was recorded 72 h after the treatment only in leaves of soybean plants treated with the highest concentration of *T. serpyllum* aqueous extract (Fig. 1).

Allelochemicals might directly inhibit oxidizing enzymes in some way, leaving the plant vulnerable to oxidative damage, similar to the reports of Qian et al. (2009). It has been documented that GSH can remove ROS and also protect proteins from the oxidation of protein thiol groups into denaturation (Noctor, 2002). *T. serpyllum* aqueous extract with concentration of 0.1% induced production of GSH in leaves of soybean plants 24 h, and in leaves and roots 72 h after treatment (Fig. 2). Also, 24 h after the treatment with *T. serpyllum* aqueous extract with concentration of 0.2%, roots of soybean had highest level of GSH content (Fig. 2).

Conclusion

In conclusion, our results showed that low concentration of *Thymus serpyllum* L. aqueous extract did not induce lipid peroxidation in soybean seedlings, while the highest concentration used (0.2%) enhanced lipid peroxidation process 72 h after the treatment. Furthermore, concentration of 0.1% of *T. serpyllum* aqueous extract stimulated GSH accumulation in soybean seedlings which propose its possible stimulative effect on antioxidant system.

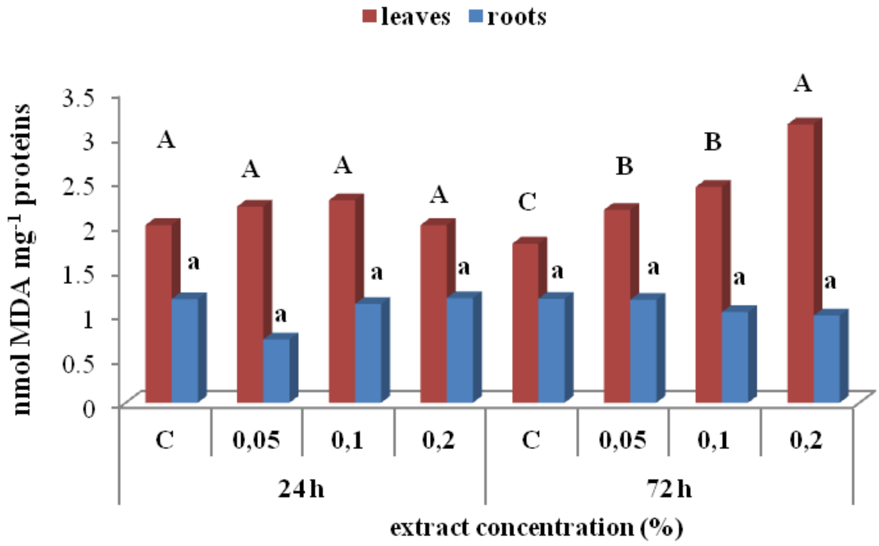

Fig. 1. Lipid peroxidation level in leaves and roots of soybean seedlings 24 and 72 h after treatment with different concentrations (%) of *T. serpyllum* aqueous extracts (v/v) and in control (C). Values marked with different letter differ significantly at P<0.05 (Duncan's test).

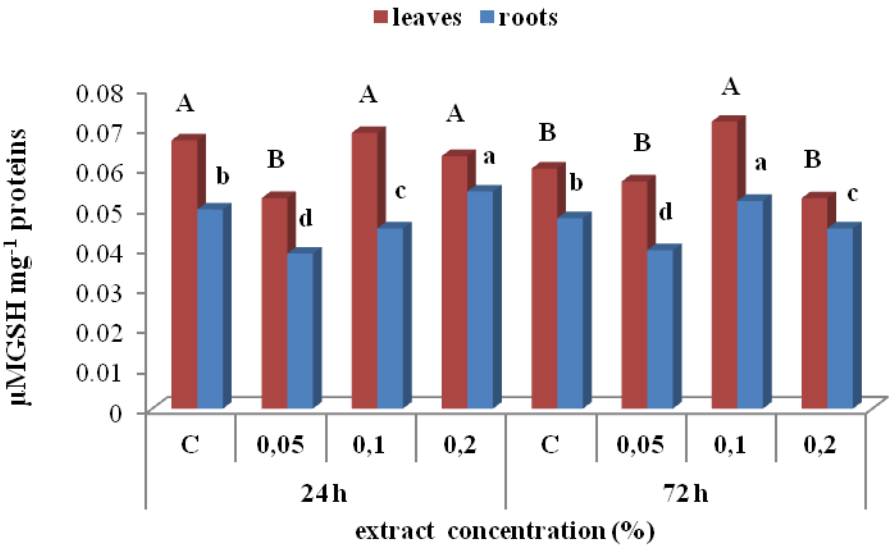

Fig. 2. Reduced glutathione content in leaves and roots of soybean seedlings 12 and 48 h after treatment with different concentrations (%) of *T. serpyllum* aqueous extracts (v/v) and in control (C). Values marked with different letter differ significantly at P<0.05 (Duncan's test).

Acknowledgements. This study was carried out within a project of the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant N^{o} TR-31022.

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