INFLUENCE SELENIUM NANOPARTICLES AND SODIUM SELENITE ON THE ANTIOXIDANT POTENTIAL AND YIELDS OF RED CLOVER

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Abstract: In this study, we examined the application of selenium in various forms and doses on the growth of red clover. The first experimental factor was the form of selenium – sodium selenite and selenium nanoparticles modified pork gelatin. The second experimental factor was dose selenium – 0, 2 and 20 mg/m². Sampling was conducted at an interval of 14 days. It has been shown that high doses of sodium selenite and selenium nanoparticles decrease the yield of green clover. Conversely used form of selenium had no effect on the yield and other indicators.

Key Words: Trifolium pratense L., selenium, yield, GSH, forage

INTRODUCTION
Selenium (Se) is a widely studied a trace element and its role in plant growth and physiology are well documented. At low concentrations plays a protective role in abiotic stress tolerance, while higher concentrations show phytotoxicity. Plant species differ markedly ability to scavenge and accumulate selenium. Insufficient supply of the organism that element leads to many diseases (Hasanuzzaman et al. 2014, Kaur et al. 2014, Wu et al. 2015). It is part of selenoproteins (e.g. glutathione) prevents oxidative destruction the biological diaphragms. Its deficiency causes therefore weakening the overall health status (Horký et al. 2016, Skaličková et al. 2016).

Selenium nanoparticles exhibit excellent biological activity and low toxicity (Zhang et al. 2001, Wang et al. 2007).

The tripeptide glutathione in animal and plant cells is represented in a high concentration and contributes to the elimination of free radicals. The reduced form of glutathione (GSH) in cells involved in protective and detoxification processes (Wünschiers 2012, Fajt et al. 2009).

The perennial red clover belong to the family Fabaceae and this is one of the most commonly used clover for feed purposes. It is very suitable for grazing use, because it contains high-quality and digestible protein. Deep roots allow it to grow even during the summer and drought (Graves et al. 2012).

The aim of this study was to determine the effect of foliar application of selenium in various forms and doses on antioxidant status and forage yield of red clover.

MATERIALS AND METHODS
The experiment was established as a pot experiment in climate chamber CLF PlantMaster – CLF Plant Climatics (Wertingen, Germany). Mode was set to 24 °C day temperature, 20 °C night temperature, humidity of 65% throughout the day, duration of sunshine 12 hours, light intensity of 300 µm/m/s. To provide the experiment was chosen species red clover (Trifolium pratense L.), two forms of selenium (sodium selenite and selenium nanoparticles modified pork gelatin). It was used as substrate grass substrate with silica sand. To each container was weighed 500 g substrate, 0.05 g of red clover.
seed and supplemented by additional 50 g of substrate. The experiment was maintained only watering demineralized water as needed, until the cover is fully engaged.

37 days after the establishment of the experiment was performed application of solutions of various concentrations outside climate chamber (Table 1). On the day of application were prepared solutions and with the help of hand sprayer applied to individual groups of homogeneous test plants. The plants were then left for 24 hours after application in standard conditions (22 °C, 70% relative humidity) and then displaced back to climate chamber.

Table 1 Forms and dose applied solutions (mg/m²)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Dose of Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>A – Sodium selenite</td>
<td>2</td>
</tr>
<tr>
<td>B – Sodium selenite</td>
<td>20</td>
</tr>
<tr>
<td>C – Selenium nanoparticles modified pork gelatin</td>
<td>2</td>
</tr>
<tr>
<td>D – Selenium nanoparticles modified pork gelatin</td>
<td>20</td>
</tr>
</tbody>
</table>

The first samples were taken on the day of application of selenium. Other sampling took place 14 and 28 days after application. Overhead phytomass from each container was trimmed to 1 cm stubble height, weighed and immediately after sampling deep-frozen. The vegetation was nursed only watering demineralized water evenly in all groups. Continuously throughout the duration of the experiment was weeding vegetation.

To optimize the determination of the reduced form of glutathione (GSH) was used flow injection analysis with electrochemical detection system (FIA-ED). GSH was determined by HPLC-ED (Potěšil et al. 2005).

The results were processed in the STATISTICA 10 CZ (Czech Republic) using a multifactor analysis of variance ANOVA. Differences were considered significant at a P < 0.05.

RESULTS AND DISCUSSION

Yield of green forage clover was the day of application and the first collection of all experimental groups balanced. 14 days after application of selenium yield increased independent of dose and form of selenium. 28 days after application is observed a lower yield of green forage in groups B and D with selenium dose of 20 mg/m², compared to the control group. Conversely application selenite and selenium particles at a dose of 2 mg/m² had no significant effect on the yield of red clover (Figure 1).

Figure 1 Effect of selenium in various forms and dose on the yield of red clover (g/m²)

Legend: A – selenium as selenite 2 mg/m²; B – selenium as selenite 20 mg/m²; C – selenium as selenium nanoparticles 2 mg/m²; D – selenium as selenium nanoparticles 20 mg/m². Error bars indicate 95% confidence interval.
**Figure 2** Effect of selenium in various forms and dose on the GSH of red clover (µM)

![GSH Content Graph](image)

Legend: A – selenium as selenite 2 mg/m²; B – selenium as selenite 20 mg/m²; C – selenium as selenium nanoparticles 2 mg/m²; D – selenium as selenium nanoparticles 20 mg/m². Error bars indicate 95% confidence interval.

GSH content as indicators of antioxidant potential of the organism is illustrated in Figure 2. For the control group is the marked reduction in value between the first and third collection. Generally observed in all other groups, reduction of GSH values in time. It does not depend on the dose used or the form of selenium, after application temporarily GSH values slightly increase (P> 0.05), then there is a reduction values. A GSH level varies depending on the selenium content in forage (Figure 3). After application increases the value of selenium in the phytomass and over time these values decrease as selenium in the organism metabolized and stored in the root system.

**Figure 3** Effect of selenium in various forms and dose on the selenium contain of red clover (µg/g)

![Selenium Content Graph](image)

Legend: A – selenium as selenite 2 mg/m²; B – selenium as selenite 20 mg/m²; C – selenium as selenium nanoparticles 2 mg/m²; D – selenium as selenium nanoparticles 20 mg/m². Error bars indicate 95% confidence interval.
CONCLUSION
Our results show that selenium affects the selenium content in the biomass and the value of GSH in the first two weeks after application. It was also observed decrease of the yield on treated crops of selenium. Used form of selenium had no significant impact on the evaluation indicators (yield, GSH and selenium content). It can therefore be understood selenium nanoparticles as an alternative form of selenium in nutrition of plants.

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REFERENCES