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ESTIMATION OF ANTIOXIDANT ACTIVITY OF THE SOILS FROM AN ORCHID RESERVE

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A b s t r a c t. Taking into account high organic matter content in the orchid reserve soils of the Roztoczański National Park, the present authors tried to adapt the method worked out by Rice-Evans and Miller to estimate a soil extract antioxidant activity. This method is used to compare antioxidant activity of complex substances with various organic compounds. The method corroborated the occurrence of antioxydation substances in the tested soil samples.

K e y w o r d s: orchid, antioxidant activity, reserve, the Roztoczański National Park.

INTRODUCTION

The Roztoczański National Park has very rich and varied flora. It is the result of high physiographical variability of the park area and its varied relief, solum lithology, water relations, soils and topoclimate. Among the protected plant species of the park, there are orchids, a foristic curiosity of the park [4,7].

Orchids belong to one of the most special plants of the world. They are admired for their diversity of form, shape, colour, flower smell, way of living and pollination. The majority of species from the orchidaceous family is very sensitive and has high and very specific habitat requirements and various ways of adaptation to living conditions. Any forms of human impact provoking changes of the stabilised conditions determine rapid orchid extinction. Orchids are calciphilous plants growing in subhumid places, on very fertile but not leached soils [7]. Most often they occur on marls or lime rendzina. The place where magnificent orchid species can be found in the Roztoczański National Park is the nature reserve - Maziarki.

The aim of the study was to measure antioxidant activity of the extracts prepared from the soil samples taken from the orchid reserve, from the areas with correct orchid growth, single individuals where the orchids have not been observed before.

MATERIALS

Collection of the soil samples

Samples were collected from the topsoil of the Roztoczański National Park orchid reserve, from the areas with correct orchid growth, single individuals where the orchids have not been observed before. Antioxidant and chemical-biological properties were determined in the average mixed samples. Soil extracts prepared at the ratio 1:2 (10 g of soil + 20 ml phosphate buffer - pH 6.8) were used for the analyses. The soil solutions were homogenised and filtered by filter paper. The filtrate in the amount of 200 µl was used for the analyses.

Determination of the selected soil properties:

- soil pH in H_2O and 1 M KCl by the electrometric method [8];
- organic carbon content by the Tiurin's method;
- calcium carbonate according to the Scheibler's method [8].

Determination of the antioxidant properties

Taking into consideration the high content of organic substances in the investigated soil, the authors tried to adapt the method used for the estimation of soil extract antioxidant activity worked out by Rice-Evans and Miller [1,6]. The method by Rice-Evans and Miller is used to compare antioxidant activity of complex substances containing various organic compounds. It is a very sensitive method that allows to determine antioxidant activity in the soil extracts with low pH. In the control sample with 20 mM AAPH and 150 µM ABTS in the phosphate buffer at the temperature of 37 °C, radical cations of $ABTS^{0+}$ are formed. An increase of absorption to the $ABTS^{0+}$ concentration is observed [6].

The present authors adapted a method worked out by Rice-Evans and Miller for the estimation requirements [6]. The method of antioxidant activity estimation consist in the incubation of ABTS (2'2'-azino-di-[3-ethylobenzothiazoline sulphonate]) and AAPH (2'2'-azobis[2-amidinopropane]). The product of the reaction is a radical of $ABTS^{0+}$. This compound has a specific colour which is stabile at the wavelength of 660 nm. A compounds with antioxidant properties that is added to the reaction mixture, inhibits formation of the radical cation of $ABTS^{0+}$. The percentage inhibition of the above reaction is proportional to the antioxidant compound concentration in the tested sample. The percentage of inhibition is calculated from the following formula:

% inhibition =[(Absorption of control sample - Absorption of tested sample)/ Absorption of control sample]x100.

The above method can be applied to estimate a complete antioxidant potential in the extracts produced from the yeast cells exposed to the action of ionising radiation in order to investigate changes during fruit juice storage and clinic tests of blood plasma of prematurely born children, new-born babies and their mothers [5].

Reagents: ABTS (2'2'-azino-di-[3-ethylobenzothiazoline sulphonate]) Sigma, AAPH (2'2'-azobis [2-amidinopropane]) by Poly Science, other reagents by POCH.

Equipment: Absorption was measured by the Beckman DU 68 spectrophotometer.

RESULTS AND DISCUSSION

The tested soil samples from the orchid reserve had a slightly acid pH value. Acid pH (pH in 1 M KCl - 4.4, in H_2O - 5.2) was found in the soil samples taken from the areas where orchids did not exist [7]. We can conclude that pH exerts a significant influence on the orchid occurrence in the investigated reserve. The soil pH is directly related to the presence of calcium carbonate and organic carbon in the soil environment. At higher pH levels, higher content of $CaCO_3$ was observed. However, the amount of $CaCO_3$ was differentiated in the subacid horizons. A considerable quantity of $CaCO_3$ in the topsoil could contribute to correct growth of orchids in spite of the subacid top horizons. The studied soil samples had similar amounts of organic carbon (Table 1). It was observed that orchids usually develop in the soil environments with high sorptive capacity. It is related to the possibilities of nutrient assimilation in the complexes.

Table 1. Same properties of the studied soils

Soil samples taken from:	pH		Corg. (%)	$CaCO_3$ (%)	Sorptive capacity (T) cmol(+)/kg
	H_2O	1 M KCl			
Areas with shortage of orchids	5.2	4.4	7.21	0.08	17.63
Areas with single individuals	6.3	6.2	7.06	10.76	27.48
Areas with great amount of orchids	6.5	5.9	6.58	40.98	26.78

In the tested samples with a known soil extract concentration and reagents, as in the control sample, inhibition of forming radical cation $ABTS^{0+}$ by the antioxidant substances is observed. A decrease in the absorbance of the tested samples in relation to control is proportional to the amount of antioxidant substances.

The maximum inhibition of free radical reaction was observed in the soil extracts made of the soil samples taken from the areas with higher amounts of orchids (Table 2). It was 37.5% after 60 min. After the same time period, the soil extract made of the soil samples from the areas where only single orchid individuals can be found, inhibited the above reaction by 20.5% and soil extract made from the areas where orchids did not exist - by 11.5%.

Table 2. Percentage of free radical reaction inhibition in the soil extracts

Soil samples taken from:	Percentage of free radical reaction inhibition in time (minutes)					
	15	30	45	60	75	90
Areas with shortage of orchids	4.5	5.5	8.5	11.5	1.2	1.1
Areas with single individuals	15.8	17.5	20.1	20.5	18.2	10.5
Areas with great amount of orchids	30.0	31.0	34.1	37.5	30.8	24.5

ONCLUSIONS

1. Orchids are calciphilous plants and require proper soil pH.

2. Sorptive soil abilities exerted a significant influence on the orchid growth. The higher this value, the higher the amount of orchids.

3. The applied method, worked out by Rice-Evans and Miller, allowed to estimate the occurrence of the antioxidant substances in the tested soil samples.

4. The maximum inhibition of free radical reaction in the soil extracts made of the soil samples taken from the areas where there were a lot of orchids was observed after 60 minutes and amounted to 37.5%.

5. High amount of organic compounds in the soil samples taken from the areas where there were a lot of orchids was a decisive factor for the maximum inhibition of the free radical reaction.

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