BIOCHEMICAL INDICES OF PROOXIDANT-ANTIOXIDANT PROCESSES IN *CALENDULA OFFICINALIS* L., GROWN UNDER THE INFLUENCE OF GROWTH BIOSTIMULANTS

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Abstract. The effect of plant growth biostimulants «Vermymag», «Vermyiodis» and «Vermystym» on the content of enzymes of antioxidant protection and metabolites of the prooxidant-antioxidant system of *C. officinalis* flowers of the cultivated variety «Poliova Krasunia» cultivated in the soil and climatic conditions of the Precarpathian region has been analyzed. It was shown that applying biostimulants during cultivation of *C. officinalis* flowers contributed to the increase ($p<0.05$-0.01) of antioxidant enzymes activity compared with control plants. In particular, the superoxide dismutase and catalase activity was higher with applying biostimulants «Vermymag» and «Vermyiodis». The biostimulant «Vermymag» had a positive influence on the peroxidase activity, contributing to the increase of enzyme content up to 21% comparing with the control. The content of ascorbic acid increased under the influence of all biostimulants in plant cells. The use of stimulants positively influenced the redox state of *C. officinalis* cells, contributing to suppressing lipoperoxidation processes and increasing the resistance of plants to the action of stress factors. It is found out that growth biostimulants promote the balance of prooxidant-antioxidant processes in *C. officinalis* plants.

Keywords: *Calendula officinalis* L., growth biostimulants, antioxidant enzymes, ascorbic acid.

DOI: http://dx.doi.org/10.23856/3414

Introduction

As a result of the growth of the degenerative influence of anthropogenic factors on the state of natural resources, there was a need for quality medicinal plant raw material. In the complex therapy of many diseases, the extracts of *Calendula officinalis* are used due to the high content of natural antioxidants and other biologically active substances in the flowers of the plant. In Ukraine there is no range *C. officinalis*, the plant is only cultivated for medical needs in specialized farms, in the gardens and on the plots of land *(Lupak et al., 2018)*. The use of biostimulants during plant cultivation contributes to the growth and increase of their stress resistance, activating the antioxidant potential of plants *(Terek, 2018).*

Within our previous researches *(Lupak et al., 2018)* we established the positive influence on the growth and development of *C. officinalis* plants, grown with applying biostimulants «Vermymag» (the former name «Vermybiomag»), «Vermyiodis», and «Vermystym» in the conditions of the Precarpathian region.

The purpose of the study was to explore the content of enzymes of antioxidant protection and metabolites of the antioxidant-prooxidant system of *C. officinalis* cv. «Poliova Krasunia» cultivated in soil and climatic media under the influence of growth biostimulants «Vermybiomag», «Vermyiodis» and «Vermystym». It has been established the positive influence of biostimulants on morphometric indices, crop capacity, and content of some biologically active substances of the inflorescences of *C. officinalis*. It was also found out that biostimulants contribute to the increase of integral antioxidant activity *(Lupak et al., 2017)*, that is determined with physiological and biochemical processes of prooxidant-antioxidant activity of the plants' system.

That is why the purpose of our research was to explore the content of enzymes of antioxidant protection and metabolites of prooxidant-antioxidant system of *C. officinalis* plants cv. «Poliova Krasunia», cultivated in soil and climatic conditions of the Precarpathians under the influence of growth biostimulants «Vermymag», «Vermyiodis» and «Vermystym».

Materials and methodology of the research

Plants of *C. officinalis* cv. «Poliova Krasunia» were cultivated on the educational area of Drohobych Ivan Franko State Pedagogical University during 2015-2017 of the Precarpathian region under the influence of growth biostimulants «Vermymag», «Vermyiodis» and «Vermystym».

Biostimulants have being applied twice into experimental areas – in phenological phases of shoots and budding of plants at a rate of 5 l/ha. The experimental sites where the biostimulants were not applied during plant cultivation were used as the control ones. The accounting area of the plot is 10 m^2.

The activity of superoxide dismutase (SOD) was measured by the ability of the enzyme to inhibit photochemical reduction of nitrosine tetrasol (NTS). The incubation mixture contained 0.15 M Na-phosphate buffer (pH 7.8), 1×10^{-6} M EDTA, 0.4×10^{-3} M NTS, 1.8×10^{-6} M phenazine methsulphate, 0.1×10^{-6} M NADH. The incubation of the samples was being carried out for 10 minutes in the dark at 20°C. The optical density of the samples was measured at SF-2000 at 540 nm. The unit of activity of SOD took the amount of enzyme that is capable of suppressing the reaction of photosynthesis of NTS by 50% *(Chevari, 1985).*

The activity of catalase was measured by the spectrophotometric method (SF-2000, OKB «Spectrum», RF), which is based on the ability of hydrogen peroxide to form a stable colored compound with ammonium molybdate. The activity of catalase was expressed in mmol H_2O_2 / min per 1 mg of protein. To prepare the cell extract, the selected material was homogenized with an extraction buffer containing 0.1 M phosphate buffer, pH 6.8, 20% glycerin, 30 mM dithiothreitol, 0.1% polyvinylpyrrolidone (PVP) *(Doliba et al., 2010)*. The homogenate was cooled to + 40 ºC and centrifuged at 10000 g for 15 minutes. The supernatant served to determine the content of catalase and other enzymes and protein. Protein was determined with the help of Bradford's method (*Bredford, 1976).*

The activity of peroxidase was determined by the amount of colored peroxidase oxidation product produced by amino antipyrin at 510 nm *(Mogharrab, 2007).*

Determination of ascorbic acid (AA) content. The content of AA in extracts of *C. officinalis* inflorescences was determined by Murry using the Tilmans reagent (2,6-dichlorophenolindophenol), the aqueous solution of which under the influence of AA was discolored *(Musiienko, 2001). The extract of fresh inflorescences* was prepared by grinding 5 g of vegetative material in a porcelain cup at the availability of 2% metaphosphoric acid and bringing the volume to 100 ml with the same acid. The homogenate was centrifuged at 700 g. In the test samples, 3 ml of extract of fresh raw material and 0.3 ml of 0.025% solution of 2,6-dichlorophenolindophenol were added. The stopwatch was immediately switched on and after 35 s photometrised at 530 nm in a cuvette with a working length of 1 cm against 2% acid. The control was a sample containing 3 ml of 2% acid and 0.3 ml of dye. The change in the intensity of the color of the test solution is proportional to the amount of ascorbic acid. In order to calculate the number of AA, they built a calibration diagram.

The content of **malondialdehyde** (MDA) determines the methodology, which works on the detection of colored product at 532 nm, which is generated by the interaction of MDA with 2-thiobarbituric acid *(Musiienko, 2001)*. 0.5 g of the plant material are homogenized with 3 ml of water, 3 ml of 20% TCVA are added to the homogenate and again homogenized. From the obtained homogenate in 2 samples, 2 ml was taken. To one of them (control) 2 ml of 20% TCVA are introduced. To another (experimental) sample, 2 ml of 0.5% TBA (in 20% of TCVA). Samples were incubated for 30 minutes in boiling water baths, cooled and centrifuged for 10 min at 1000 g. The optical density was determined at 532 nm.

Statistical analysis of experimental data. The experiments were being carried out at three biological and five analytical repetitions. For each sample of indicators, the mean arithmetic and quadratic value (M), the standard error of the average (m), Student's coefficient and reliability were determined. Statistical data was processed using the Microsoft Statistica 6.0 software, the discrepancies between the samples were considered significant at $p \leq 0.05$.

Results of the study and their discussion

The universal property of plants is their ability to induce enzymes with antioxidant protection under adverse conditions that allow the cell to avoid toxic effects of free radicals *(Terek, 2018)*. We analyzed the content of enzymes of antioxidant protection (superoxide dismutase, catalase, peroxidase) and low molecular weight metabolites (ascorbic acid and peroxide oxidation products of lipids) in *C. officinalis* «Poliova Krasunia», cultivated in the Precarpathian region under the influence of growth biostimulants.

Superoxide dismutase is one of the key enzymes of the antioxidant system of cells and tissues and carries the protective role of the cell from active forms of oxygen *(Zhang et al., 2015)*. Only this enzyme catalyzes the dismutation of the superoxide anion radical O^2 to O^2 and H^2O^2, thus regulating the intracellular concentration of free oxygen radicals. *(Kolupayev, 2017)*.

As a result of the study of the effect of growth biostimulants «Vermymag», «Vermyiodis» and «Vermystym» on the activity of SOD in extracts of various organs of plants *C. officinalis*, it is found (Fig. 1) that it is significantly higher ($p<0.05$) by 15.4 - 22.9% in plants grown under the influence of stimulants «Vermymag» and «Vermyiodis» compared to control plants. The exact difference between these experiment variants was not revealed. At the 7th day the content of SOD increased after the 2nd introduction of «Vermymag» from 21,1% up to 24,3 %, «Vermyiodis» from 18.6% up to 19.9%. At the 14th day after applying these stimulants the specific activity of SOD increased from 17,9% up to 19,1 % and 15,4 – 17,6 % accordingly. «Vermystym» did not influence on the enzyme content.

It was found out that the specific activity of SOD varies in different organs of plants. In particular, the highest its content was revealed in the flowers of the plant, what is higher by 25,2% and 19,3% than in roots and stems accordingly.

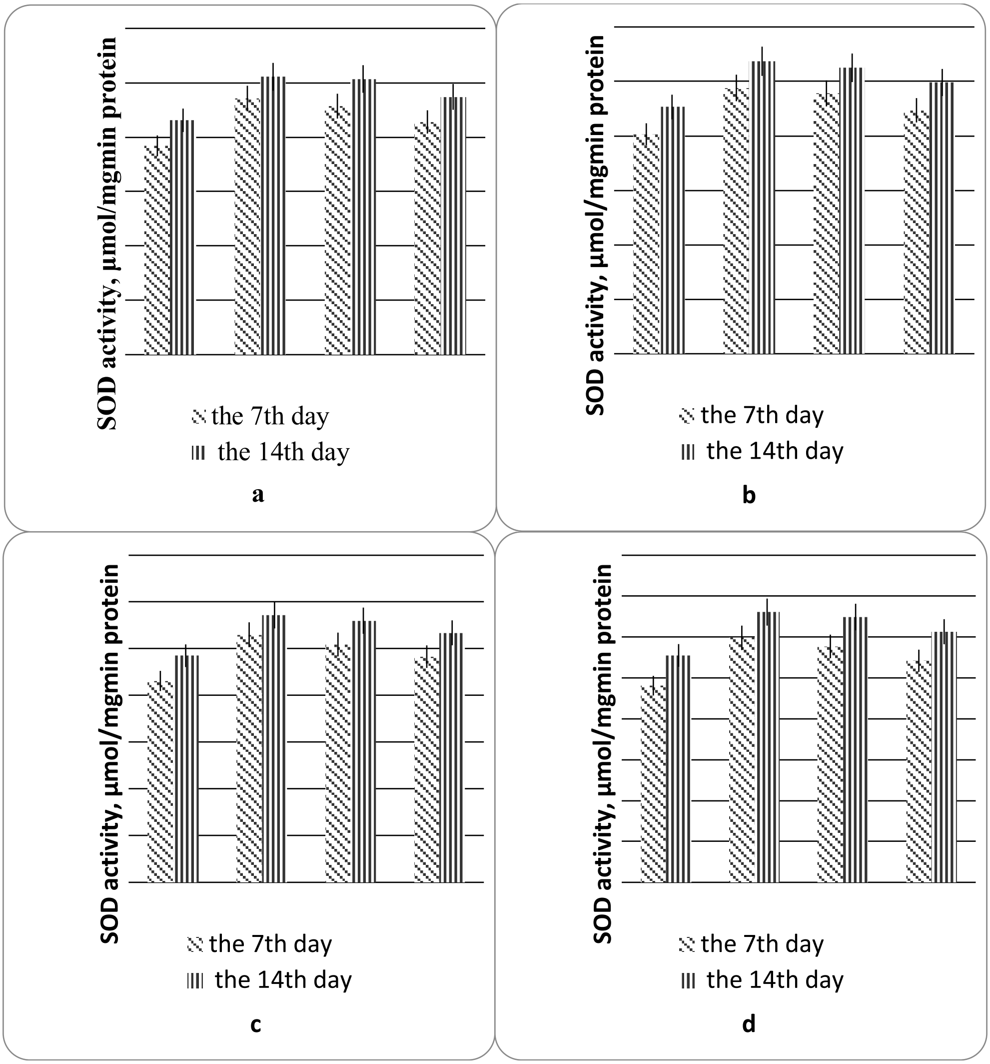

Fig. 1. Specific activity of SOD in *C. officinalis* plants
a – roots, b – stems, c – leaves, d – flowers;
1 – control, 2 – «Vermymag», 3 – «Vermyiodis», 4- «Vermystym»

Taking into account that as a result of the work of the SOD hydrogene peroxide is generated, the neutralization of which is provided by catalase and peroxidase, therefore we have determined the activity of these enzymes in the flowers of the investigated *C. officinalis* plants, since the flowers are important generative organs of plants and depot of important BAS-metabolites designed to maintain stability and creation of favorable conditions for germination of seeds. In addition, it is the flowers of these plants used in medical practice, and their biologically active substances determine the effectiveness of treatment.

It was found that both enzymes are present in trace amounts, which may indicate a high equilibrium of oxidative-reduction reactions in the studied plants (Fig. 2).

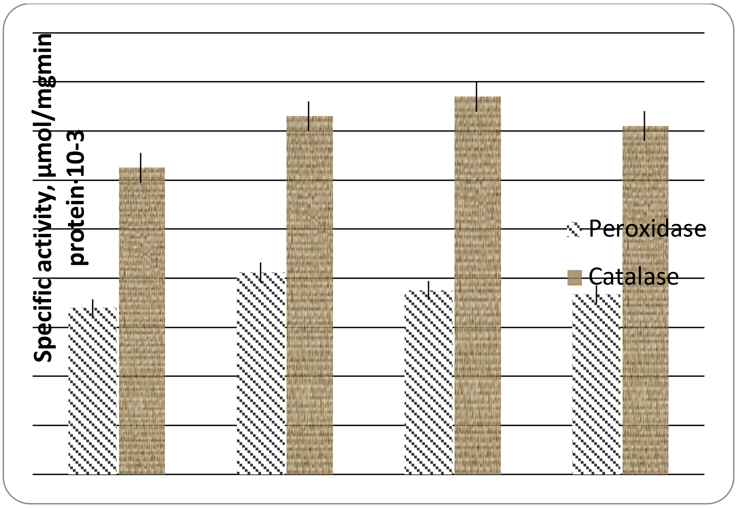

Fig. 2. Specific activity of peroxidase and catalase in the extracts of flowers of *C. officinalis* cultivated variety «Poliova Krasunia»

Inflorescences of *C. officinalis* plants grown under the action of the «Vermymag» growth biostimulant are characterized by a higher ($p<0.05$) peroxidase activity of 21% compared with control. Our researches are consistent with literary data *(Makogonenko et al., 2018)* that revealed the increased activity of peroxidase in sunflower seedlings by the action of the growth regulators «Regoplant» and «Stympo» compared with the control, which contributed to the increase of the transfer of oxygen from certain substrates to peroxide hydrogen.

Under normal physiological conditions, catalase regulates hydrogen peroxide in the body, preventing its toxic effects play an important role in aging plants. In plant cells, it is localized in peroxisomes and cytosols *(Doliba et al., 2010; Nasrabadi, 2008)*. It has been established that in the inflorescences of *C. officinalis* plants grown under the action of growth biostimulants, the activity of catalase is significantly higher than control. It was shown that during the action of the «Vermyiodis» biostimulant, the activity of the enzyme was 23.2% higher than control, while «Vermymag's» exposure was 16.8%, the effect of «Vermystym» was insignificant.

Stress factors, with which plants constantly have to interact, lead to the destruction of membranes. One of the main indicators of this process is the changes in peroxide oxidation of lipids. An important indicator of the balance of the antioxidant state and the level of peroxide oxidation of lipids in organisms is malonic dialdehyde (MDA) *(Baranov et al., 2018)*. Therefore, we determined its content in extracts of inflorescences of *C. officinalis,* grown for the influence of biostimulants. Inflorescences of *C. officinalis* plants grown under the action of biostimulants of growth are shown to be characterized by lower content of TBARS by the formation of MDA compared with control, which contributes to better plant protection. In particular, according to the actions of «Vermymag», the content of TBC-active products decreased by 20.8% in terms of control, for «Vermyiodis'» influence – by 19.13%, and for «Vermystym», by 15.1% (tabl.). The obtained results are consistent with literary data that reveal the reduction of LHP in the fir-needle of *Pinus sylvestris* L. plants by the action of growth regulators *(Baranov et al., 2018)*.

Table 1

The influence of biostimulants on the content of MDA and ascorbic acid in the extracts of inflorescences of *C. officinalis* plants (M±m)

Variants of the research	Content of MDA, nmol/g abs. of dry mass of inflorescences	Content of AA µg/g abs. of dry mass of inflorescences
Control	21,17±0,98	1,139±0,06
«Vermyiodis»	17,12±0,71 **	1,406±0,09 *
«Vermymag»	16,76±0,79**	1,375±0,08*
«Vermystym»	17,98±0,82*	1,345±0,06*

Note: *, ** - the probability of the difference between the control and the experiment variant (* - p<0.05, ** - p<0.01)

It is known that ascorbic acid (AK) is an important low molecular weight antioxidant, which is an indicator of the orientation of oxidation-reducing processes, and determines the resistance of plants, since one of the main functions of AK is the restoration of free radicals and minimization of oxidative stress disturbance *(Bilchuk et al., 2012; Horemans et al., 2000; Kolupaev et al., 2017).*

It was found out that inflorescences of *C. officinalis* plants grown under the influence of growth biostimulants have higher content of AA comparing to the control (p<0.05) from 18,1 up to 23,4 %, that contributes to better protection of plants. It was shown that somehow higher indices were obtained in the variant with applying «Vermymag».

Conclusions

1. It was found out that activity of SOD in the extracts of various organs of plants of the flowers of *C. officinalis* is significantly higher (p<0,05) from 15,4 up to 22,9 % in plants, grown under the influence of «Vermymag» and «Vermyiodis» comparing to control.
2. Extracts of inflorescences *of C. officinalis* plants, grown under the action of «Vermymag» are characterized by higher peroxidase activity comparing to control. It has been established that in the inflorescences of *C. officinalis* plants grown under the influence of growth biostimulants «Vermyiodis» and «Vermymag», the activity of catalase is significantly higher than control.
3. It was shown that inflorescences of *C. officinalis* plants*,* grown under the action of growth biostimulants are characterized significantly lower (p<0.05-0.01) content of TBARS after formation of MDA comparing to the control, that contributes to better protection of plants.
4. It was found out that inflorescences of *C. officinalis* plants grown under the influence of growth biostimulants have higher content of AA comparing to the control (p<0.05) from 18,1 up to 23,4 %, that contributes to better protection of plants.
5. The use of stimulants positively influenced the redox state of *C. officinalis* cells, contributing to suppressing lipoperoxidation processes and increasing the resistance of plants to the action of stress factors. It is found out that growth biostimulants promote the balance of prooxidant-antioxidant processes in *C. officinalis* plants.

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