# DETERMINATION OF THE INFLUENCE OF GROWTH CONDITIONS ON THE AMOUNT OF ANTIOXIDANT SYSTEM ENZYMES (SUPEROXIDE DISMUTASE, PEROXIDASE) IN ARTEMISIA ANNUA LEAVES

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***Abstract.*** *The article describes the results of experiments aimed at determining the differences in the amount of enzymes of the antioxidant system (AOS) in cultivated and wild Artemisia annua plants.*

***Keywords:*** *Artemisia annua, antioxidant, active forms of oxygen, oxidative stress, superoxide dismutase (SOD), peroxidase.*

**Introduction.** The flora of Uzbekistan is very rich in medicinal plants used for the production of medicinal preparations in the pharmaceutical industry. Currently, more than 400 cultivated and wild medicinal plants growing in Uzbekistan have been studied and described (Kurmukov A. G., 2014). The annual production of technical, food and medicinal raw materials is 300 tons, the range of harvested plants exceeds 30 species (Shakarov J., Idriskhodjaev U., 2014). It is known that the effectiveness of medicinal plants depends on the composition of a number of chemical compounds that determine their therapeutic effect. Recently, the cultivation of medicinal plants in industrial plantations has started to develop actively in Uzbekistan, due to the support provided by our state. A targeted program for the creation of industrial plantations and a list of medicinal plants recommended for cultivation are being developed. Also, the decree of the President of the Republic of Uzbekistan Sh. Mirziyoyev No. PF-5032 dated May 3, 2017 stipulates the study of the introduction and cultivation of medicinal plant materials.[4]

Plants need light, water, carbon and minerals to grow and develop. However, various types of stress hinder the development and cause the yield to decrease from the optimal level. The influence of unfavorable factors helps to activate adaptation mechanisms at the morphological, physiological, biochemical and molecular level. Among them are active forms of oxygen (AFO), accumulation of plant hormones, changes in the oxidation-reduction state and fluxes of inorganic ions. AFO - it is a group of free radicals, reactive molecules and ions derived from O2. Approximately 1% of the O2 consumed by plants is directed to the formation of AFO in the loci of various organelles, such as chloroplasts, mitochondria, and peroxisomes.[3]

AFO has harmful and beneficial effects depending on its concentration in plants. A high concentration of AFO damages biomolecules, while at a low, moderate concentration, it acts as a second messenger in intracellular signaling cascades that ensure several reactions in plant cells. Active forms of oxygen (ROS) are highly reactive chemical molecules formed due to the acceptance of an electron by O2, which include peroxide, superoxide, hydroxyl radical, singlet oxygen, and alpha-oxygen. Under normal conditions, excess CSF can be scavenged by various antioxidant defense mechanisms. Antioxidants feed on radicals and turn them into stable compounds and at the same time maintain their stability. The cleaning mechanisms of AFO can be divided into two types: enzymatic and non-enzymatic antioxidant protection systems, which

neutralize free radicals. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate-glutathione (AsA-GSH), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDH) and glutathione reductase (GR) enzymes. Ascorbate (AsA), glutathione (GSH), carotenoids, tocopherols and phenols serve as non-enzymatic antioxidants inside the cell.[1]

**Oxidative stress** is a complex chemical and physiological phenomenon that develops in higher plants as a result of excessive production and accumulation of active oxygen forms (AOF) as a result of all biotic and abiotic influences. Recent data clarified the "origin" of oxidative stress in plants and showed that, in addition to chloroplasts, mitochondria and peroxisomes, AOF is synthesized by NADFH oxidases and peroxidases. AOF damages all the main bio-polymers of plant cells, as a result of which their activity is disturbed.[2] They activate Ca 2+-permeable and K+-permeable cation channels of the cell membrane and cause programmed cell death.

It is known that the qualitative and quantitative content of biologically active substances in medicinal plants depends on the growing conditions. As a result of adverse environmental factors, an increase in the amount of AOF in plants can cause a change in the quality and quantity of biologically active substances, which can cause a change in the medicinal properties of medicinal plants. Many works are devoted to the study of the biological activity of extracts of medicinal plants grown in different conditions in living organisms (Goryachkina E.G. et al., 2012; Murside A.D. et al., 2014; Jimoh M.O. Et al., 2019), but insufficient attention has been paid to the influence of environmental conditions on biochemical processes in plants.

Taking into account the above points, we aimed to determine the amount of AOS enzymes in cultivated one-year wormwood - *Artemisia annua* and wild one-year wormwood - *Artemisia annua* and to determine the relevant differences.

**Material and methods**. One of the medicinal plants belonging to the Asteraceae family, one-year wormwood (*Artemisia annua*) was selected as the object of the study. The amount of SOD and peroxidase was determined from the leaves of cultured one-year wormwood growing in A.Temur avenue, Tashkent, and wild one-year wormwood growing in Bildirsoy mountain resort, district of Bustonlik, region of Tashkent.

**Superoxide dismutase** activity was determined in vitro with some modifications at a wavelength of 347 nm with the inhibition of the superoxide radical in the oxidation of adrenaline in an alkaline medium (Sirota T.V., 2000). For this, in 2 ml of 0.2 M bicarbonate buffer, pH = 10.65 (adjusted by adding NaHCO3 dry reagent to 0.2 M Na2CO3 solution), 0.1 ml of 0.1% (5.46 mM) pharmacy solution hydrochloride was added, mixed thoroughly and rapidly. The optical density is determined after 30 seconds for 5 seconds at a wavelength of 347 nm in a 10 mm quartz cuvette placed in a Cary UV 60 spectrophotometer and using a Cary UV 60 (D1) spectrophotometer. Next, 0.1 ml of the enzyme source, in our case plant homogenate, and 0.1 ml of 0.1% adrenaline hydrochloride were added to 2 ml of buffer (pH = 10.65), mixed, and the optical density was measured as described above (D2 ).

To determine SOD activity, the homogenate was obtained as follows: 100 mg of plant leaves were crushed in a porcelain mortar with 1 ml of 10 mM Tris HCl (pH 7.8). The homogenate is centrifuged at 7000 g for 15 minutes at a temperature of +2, + 4 °C. The resulting supernatant is used as a source of enzymes. Adrenalin-free buffered extract solution was used as a control sample to account for the effect of the intrinsic color of extracts that absorb certain wavelengths in the visible part of the spectrum. SOD activity was calculated using the formula.

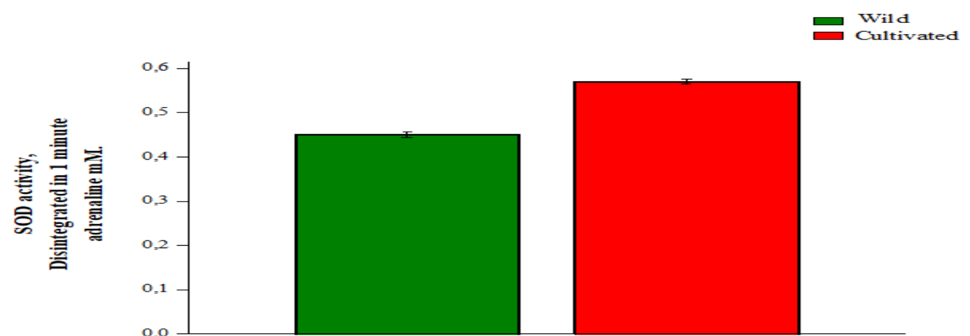
$$\mathrm{A\ (SOD)} = \frac{0"\ (\mathrm{O}) - 0"\ (K) * 54{,}6\ mM * V\ ml}{\mathrm{t}}$$

Here, A (SOD) is the activity of superoxide dismutase, adrenaline broken down per minute in mM, 0"(O) - optical density of the test sample during measurement immediately after loading, 0" (K) - optical density immediately after loading 54, 6 mM of the control sample during measurement is the concentration of adrenaline in the cuvette, V is the enzyme source in the sample (ml), t is the time of inhibition of adrenaline autoxidation, 5 min.

**Peroxidase activity** was determined by the method of Luck N. (1963). The principle of the method is based on determination of the oxidation reaction rate of benzidine under the influence of peroxidase contained in plants until the blue oxidation product of a certain concentration is formed. Preparation of benzidine solution in acetate buffer: pour 60-80 ml of distilled water into a 200 ml volumetric flask, add 2.3 ml of glacial acetic acid, and then add 0.184 g of benzidine. The flask is placed in a water bath (60 °) and, while shaking, is heated until the benzidine is completely dissolved, then 5.45 g of sodium acetate is added, shaken until dissolved. The flask is cooled to 20 °C and filled to the mark with distilled water. Preparation of plant material: 200 mg of fresh leaves were crushed in a porcelain mortar using pH 4.7 acetate buffer and transferred to a 50 ml volumetric flask using the buffer. After a 10-minute infusion with occasional stirring, the resulting peroxidase goes into solution, the extract is centrifuged at a speed of 4000-5000 revolutions. The supernatant is used to determine enzyme activity. Two cuvettes are filled with 2.0 ml of enzyme solution, benzidine solution in acetate buffer and water. Measurements are carried out in a spectrophotometer at 700 nm, with the initial value of the optical density of the test sample equal to zero. Immediately after the addition of 0.03% hydrogen peroxide, the time is recorded, and at the absorbance mark E = 0.125 or 0.250, the stopwatch is stopped and the data recorded. In this case, under the influence of peroxidase, the oxidation reaction of benzidine occurs with the formation of a blue compound. Peroxidase activity is calculated in arbitrary units from the reaction rate and is expressed for 1 g of plant material according to the formula (Luck N., 1963): $A = \frac{Eab}{mct}$

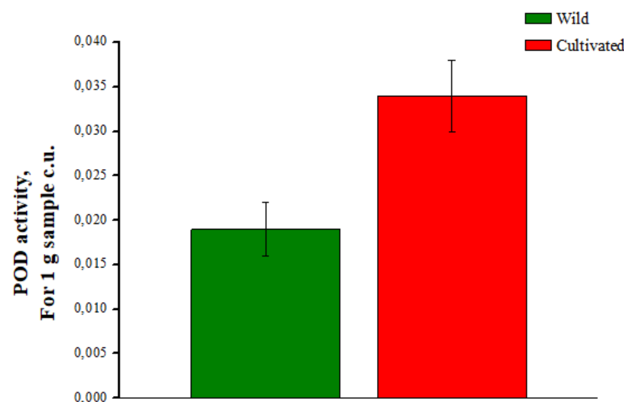
Here, A - activity of enzyme in 1 g of sample (in cube); E - absorption (0.125 or 0.250); a - volume of extract; b - degree of dilution of the extract in the reaction mixture (in the cuvette); m - weight of plant material, g; s is the thickness of the liquid in the cuvette, cm; t - time, s.

**Results.** The measurement results showed that the activity of AOS enzymes was increased in plants growing in urban conditions compared to plants in natural living conditions. SOD activity in year-old wormwood growing in urban conditions is 1.3 times higher than in mountain samples, 0.57±0.005 and 0.45±0.006 µM adrenaline/min, respectively.




**SOD activity in cultured and wild Artemisia annua leaves (µM of epinephrine cleaved in 1 min)** (n=8, p<0,05).

Peroxidase activity in annual wormwood leaves growing in urban conditions was 1.8 times higher (0.034 ± 0.004 p.p.) compared to plants growing in the mountains.




**Peroxidase activity in cultured and wild Artemisia annua leaves (s.p. per 1 g sample)** (n=8, p<0,05)

An increase in SOD and POD activity in Artemisia annua leaves was observed under urban conditions, indicating the development of a stress response in response to urban pollution, dry air, and high temperature.

**Discussion.** It is known that in response to the formation of ROS, the antioxidant system of the body is activated under the influence of stress, the data on the activity of individual enzymes are very contradictory. Thus, under the influence of unfavorable factors, SOD activity varies in different directions; in some cases, its increase is noted, and in others, its decrease, depending on the intensity of the stress factor (intensity and duration of exposure), the sensitivity of the organism, the stage of plant development, etc.

An increase in SOD and POD activity in *Artemisia annua* leaves can be observed under urban conditions, possibly indicating the development of a stress response in response to urban pollution, dry air and high temperature. This reaction has been observed in many studies. Thus, in unfavorable conditions, for example, an increase in environmental salts, a lack of regular watering, an increase in SOD activity occurs at high temperatures. At the same time, efficient activity of superoxide dismutase and peroxidase resulted in LPO levels in urban plants being maintained at the same level as plants in natural habitats.

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