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STUDY OF THE JOINT EFFECT OF MILLIMETER RANGE ELECTROMAGNETIC IRRADIATION AND CADMIUM IONS ON MALONIC DIALDEHYDE CONCENTRATION AND CATALASE ACTIVITY IN WHEAT SEEDLING CELLS

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In the work the joint effect of millimeter range electromagnetic waves and heavy metal cadmium ions on the intensity of lipid peroxide oxidation and catalase activity in wheat seedling cells has been studied. The obtained data were compared with the results received at separate influence of each of these factors. It was shown that the joint effect of electromagnetic waves and heavy metal results in improving of criteria of free-radical processes and antioxidant system activation. It was also shown that acting separately the aforementioned factors lead to sharp changes of these criteria.

***Keywords*:** wheat seedling, irradiation, heavy metal effect, malonic dialdehyde content, catalase activity.

Introduction. In nature, plant growth occurs under the influence of numerous stress-factors: temperature, humidity, strong electromagnetic irradiation, joint effect of toxic compounds and heavy metals. During the evolution living organisms have developed under the constant effect of the Earth and Space electromagnetic irradiation. Meanwhile, during the last decades the intensity of radiofrequency electromagnetic irradiation, particularly, of millimeter range electromagnetic (MM EMI) or extremely high frequencies (EMI EHF) (30–300 *GHz*), has increased [1], which affect biological systems at any level of organization [2–4]. This fact is primarily associated with scientific-technical progress, since almost all electronic devices that we use work on these frequencies. Because of the low natural background of these waves, the living organisms are not completely adapted to this physical factor. That is why studies aimed to revealed the mechanisms by which MM EMI affect biological systems are extremely actual topics. Nowadays the interaction mechanisms of MM EMI with biological systems are not fully understood. According to one vision of the mechanism of impact of electromagnetic irradiation on biological systems is tightly connected with the intensification of free-radical processes and, in particular, lipids peroxidation

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(POL) which plays an important role in plastic reconstruction of cell membranes during adaptation to changeable environmental conditions [5–7].

On the other hand, EMI EHF is not a unique external factor having an impact on living organisms. The external factors are numerous and the studies of the joint effect of two or more factors on biological systems are important and nowadays belong to small number of studies. Cadmium is one of highly toxic metals, that is found in cropland due to industrial processes and farmer practices [8, 9]. Cd is known by its phytotoxicity, which results in many morphological, physiological and biochemical problems [9–12]. Rapidly being absorbed by roots and competing with double-valence ions, cadmium suppresses the growth and development of plants and affects biomass. Heavy metals act on plant growth by different modes. One of these modes is connected to production of oxygen active forms and free radicals [13, 14]. On the other hand, it should be mentioned that Cd affects indirectly: it provokes an uptake of sulfate ions, which in turn results in suppression of photosynthesis and respiration of seedlings [15, 16].

The goal of this work is to study the joint effect of EMI EHF and Cd on the peroxide oxidation intensity of lipids and catalase activity in wheat seedling cells.

Materials and Methods. In experiments we performed, seeds and seedlings of *Triticum aestivum* L. wheat of "Bezostaya" sort 1 were used. At homogenate preparation a solution of 25 *mM* Tris-HCL buffer (pH 7.4, it contains 0.175 *M* KCl, 1 *mM* ethylenediamine tetra-acetate (EDTA), 0.5% triton X-100) was used. For sterilization of wheat seeds 0.03% potassium permanganate ($KMnO_4$) solution was used. POL was estimated by malonic dialdehyde (MDA) amount using 0.8% 2-tiobarbiturate solution [17]. MDA concentration was calculated by formula

$$C = D / \varepsilon l,$$

where D is an optical density of the studied sample; ε is extinction coefficient: $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ at 532 *nm* wavelength; l is light optic pathway length; C is MDA concentration, M. To determine of catalase activity 0.03% hydrogen peroxide solution and 4% molibdate ammonia solution were used. Catalase activity in wheat seedlings was determined by Korolyuk method [18] using formula

$$E = (A_0 - A_{\text{exp}}) V_{\text{mix}} / V_{\text{exp}} t k l,$$

where E is catalase activity; A_0 and A_{exp} are optic densities of zero and experimental samples, respectively; V_{mix} is reaction mixture volume, 3.01 *mL* (molybdate ammonia 1 *mL* is not calculated); V_{exp} is experimental sample volume, 0.1 *mL*; t is incubation time, 600 *s*; k is extinction coefficient of hydrogen peroxide, which at 410 *nm* wavelength is equal to $22.2 \cdot 10^3$ $\mu M^{-1} \cdot cm^{-1}$; l = 1 *cm*. As a unit of catalase activity the amount of enzyme was considered that in the given conditions participates to conversion of 1 *mM* hydrogen peroxide during 1 *s*.

Part of the studied samples was grown in the presence of $CdCl_2$ solution with 100 μM concentration. Optic density of the samples was measured via SF-46 spectrophotometer. As a source of electromagnetic waves G4-141 generator (USSR) was used with 37.5–53.57 *GHz* working interval and 0.6 mW/cm^2 power flux density. Stability of signal frequency was ±0.05% and the deviation of output signal frequency deviation in continuous regime of generation did not exceed 6 *MHz*. The wheat seedlings, grown in Petri's dishes, were irradiated on 50.3 *GHz* frequency. On the second day of germination the irradiation was carried out once with 60 *min* duration. In all experiments the statistical error did not exceed 5%.

Results and Discussion. Changes of MDA amount and catalase activity in the wheat seedlings at the joint effect of Cd-induced stress and EMI EHF were studied. Dependence of MDA concentration in the wheat seedling cells in the absence and presence of external factors is presented in Fig. 1.

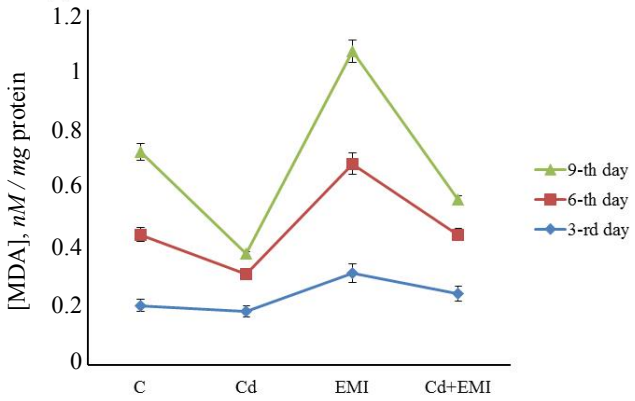

Fig. 1. Changes of [MDA] in the wheat seedling cells during the growth at the separate and joint effect of $CdCl_2$ (100 μM) and EMI (50.3 *GHz*, 60 *min*).

It can be seen from Fig. 1, in the control samples [MDA] rise with aging. In the presence of 100 μM Cd concentration in 3-day seedling cells [MDA] decreases by 10% as compared to control, in 6-day seedlings [MDA] decreases by 47.9% and in 9-day seedlings [MDA] decreases by 75%. At the irradiation by 50.3 *GHz* frequency and 1 *h* duration [MDA] increases by 55%, 54.2% and 35.7% respectively. At the joint effect of Cd with 100 μM concentration and EMI with 50.3 *GHz* frequency a following scene is observed: in 3-day seedling cells an increase of [MDA] by 20% is observed as compared to control, in 6-day seedlings [MDA] decreases by 16.7% comparing to control, though [MDA] is higher, than in seedlings treated by cadmium. In cells of 9-day seedlings [MDA] decrease is about 57.1% and in this case the enhanced value is preserved as well, comparing to the treatment by only cadmium. It follows from the obtained data that the joint effect certainly contributes to overcoming of metal-induced stress situation.

Changes of catalase activity in the wheat seedling cells at the separate and joint effects of Cd and EMI EHF are presented in Fig. 2. As it is obvious from Fig. 2, in the control samples the catalase activity increases with the growth, which permits a plant controlling the level of free-radical processes. Moreover, at the separate impact of Cd and EMI EHF in seedling cells a suppression of catalase activity occurs and at their joint influence the general scene is preserved again. It should be mentioned that in this case some improvement of the cadmium effect consequences takes place.

Thus, in 3-day seedling cells that were treated by cadmium with 100 μM concentration, a decrease of 71.9% in catalase activity is observed as compared to control, but in 6-day seedling cells it decreases by 62% and 9-day seedling cells it decreases by 47.6%. At the influence of EMI with 50.3 *GHz* frequency in 3-day seedling cells the catalase activity decreases by 1.12% as compared to control, in 6-day seedling cells it decreases by 60% and in 9-day seedling cells it decreases

63.5%. Under the joint effect of Cd and EMI in 3-day seedling cells catalase activity decreases by 48.9% compared to control, in 6-day seedling cells it decreases by 60.8% and in 9-day seedling cells it decreases by 57.8%.

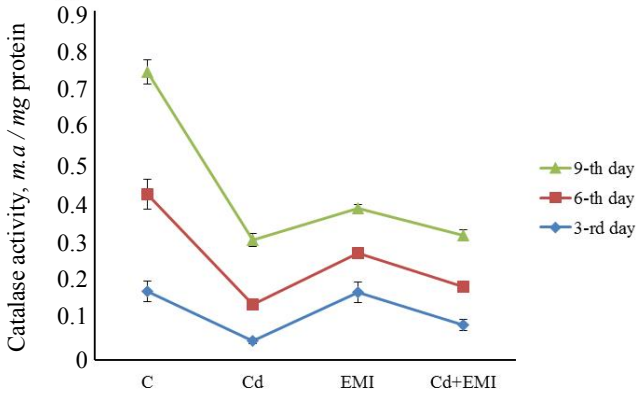

Fig. 2. Catalase activity changes in the wheat seedling cells during the growth at the separate and joint effect of $CdCl_2$ (100 μM) and EMI (50.3 *GHz*, 60 *min*).

One can notice from the obtained data that in each case the increase or decrease of MDA concentration in the seedling cells clearly correlates with the respective decrease or increase of the catalase activity, which is one of the most important enzymes of the antioxidant system of organism. It may be assumed that intensification of free-radical processes takes place not only due to the lipid peroxidation, but also due to the suppression of antioxidant system. Though, the protective mechanisms start working in the organism leading to the further activation of antioxidant system, which in turn decreases the intensity of free-radical processes. And the biological system tries to recover its homeostasis.

Conclusion. Thus, one can conclude that under the separate impact of external factors the intensity of POL and activity of antioxidant enzyme catalase are exposed to non-favorable changes for plants. If cadmium strongly suppresses the POL intensity and catalase activity, MM EMI enhances these criteria, which is not a favorable condition for wheat growth and development. Moreover, at the joint effect of MM EMI and cadmium an improvement of Cd-induced toxic state in plants is observed. On the other hand, a strong correlation between the MDA concentration and catalase activity levels is observed.

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