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ELECTROMAGNETIC IRRADIATION WITH EXTREMELY HIGH FREQUENCIES EFFECT STUDY ON LIPID FREE-RADICAL OXIDATION IN WHEAT SEEDLINGS BY CHEMILUMINESCENCE METHOD

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In this work the effect of electromagnetic irradiation by extremely high frequencies on free-radical reactions accompanied by a weak luminescence-chemiluminescence (oxidation process of lipids, intensity of spontaneous chemiluminescence) and malonic dialdehyde accumulation in wheat has been studied. It was shown that this irradiation impacts on lipids oxidation of cellular membranes of both plant seedlings and roots and leads to intensity increasing of free-radical processes. It was also shown that biological system is extremely sensitive to external effect of electromagnetic irradiation, particularly at 50.3 and 51.8 *GHz* frequencies, which correspond to own resonant frequencies of water molecule oscillations.

***Keywords*:** wheat seedlings and roots, electromagnetic irradiation water resonant frequencies, spontaneous chemiluminescence.

Introduction. Electromagnetic irradiation (EMI) with different frequencies, including extremely high frequencies (EHF) is the same of millimeter range (MM) influence on living organisms during their evolution on The Earth.

Moreover, during the recent decades the involvement of different technical devices in communicational technologies, industry and routine conditions using EMI EHF rises the effect of these exogenous factor impact on living material [1, 2]. Studies in the area of EMI EHF interaction with living organisms indicate that electromagnetic irradiation of low intensity on biological objects being on different levels of organization, differs from usual thermal impact of these waves and possesses "information-resonant" effect properties [1, 3] at which the external irradiation of organism imitates viability controlling signals produced by the organism [4]. The statement of a certain role of membranes at biological response formation to external physical effect [1, 3], as well as of water suspension role of cell [5] is generally accepted.

It has been shown that EMI of MM range significantly affects on different physiological processes of germination of seedlings, on growth and development parameters of herb organisms, whereas activation or inhibition of these processes depends on the irradiation frequency, power and duration [6, 7]. It was established

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that under the EMI effect with 50.3 and 64.5 *GHz* frequencies the increasing of activity of catalase and perpoxidase enzymes as well as of malonic dialdehide accumulation in germinating seedlings of barley and wheat occur [8]. In [9] the data of stimulating effect of EMI with 940 *MHz* on both hydrogen peroxide formation and on catalase activity of *Zeamays L.* herb are presented. As it was shown in [10] there exists dose-dependent stimulating effect of EMI on catalase activity and peroxidation level of seedling of *Triticum aestivum L.*

Moreover, primary targets of MM EMI acceptance and mechanisms of its action in biological systems are not hitherto unambiguously revealed. In vast number of studies [11, 12] it has been shown that the weak physical effects may change properties of the water solutions and though the activity of oxygen-dependent reactions in cells with formation of reactive oxygen species (ROS) (superoxide radical $O_2^{\bullet -}$, hydroxyl radical $OH^{\bullet}$ and hydrogen peroxide H_2O_2) may be changed. Interaction of the last ones with non-saturated fatty acid residues of membrane lipids induced the formation of peroxide radicals of lipids $RO_2^{\bullet}$ and as a result of their recombination non-stable tetroxide degrading with chemiluminescent (CL) light quantum yielding is formed [13, 14]. Spontaneous weak luminescence (chemiluminescence of biological tissues) is a phenomenon of radiation induced by chemical reactions of electronic excited states, gives a possibility directly to determine concentration of free radicals and dynamics of their transitions in metabolism processes [13]. Interest in free radicals is conditioned by the fact that they are participants in the important physiological processes in living organisms as well as in certain response reaction of the organism to MM EMI effect [12, 14].

The aim of this work is to study the effect of MM EMI on free-radical oxidation processes of lipids, intensity of spontaneous chemiluminescence and accumulation of malonic dialdehyde (MDA) in wheat plants.

Materials and Methods. Winter wheat (*Triticum aestivum L.*) of "Bezostaya" sort was used in experiments. To obtain ethiolaited seedlings, wheat germs were washed carefully in soap solution, treated in 0.03% solution of potassium permanganate ($KMnO_4$) and wet in water. Then they were left in water until swelling at room temperature during 12 *h*. Then the germs were put by 40 ones on wet filtered paper in Petri dishes and were couched in thermostat at 26–28°*C* for 7 days.

The irradiation of 3-day wheat herbs were carried out by G4-141 generator ("Istok", RF) and 42.2, 50.3 and 51.8 *GHz* frequencies, amplitude modulation was 10 *Hz*, average power flux density was 0.6 mW/cm^2. Irradiation was carried out through horn antenna from 20 *cm* from the object to horn basis. Irradiation duration was 1 *h*. EMI effect on intensity of free-radical processes in seedlings and roots of herb were estimated after exposition by spontaneous chemiluminescence intensity, by accumulation of one of secondary stable products of MDA [15].

Seedlings and roots (500 *mg*) of control and experimental herbs were grinded in cold porcelain mortar with 5 *mL* Tris-HCl buffer (25 *mM*, pH 7.4, containing 0.175 *mM* KCl, 1 *mM* EDTA, 0.5% triton X-100) and homogenized in Potter-Elvehaim homogenizer. The obtained homogenate was centrifuged with 4000 *rpm* 10 *min* 4°*C*. Supernatant was used in further investigations. Protein was determined by Lowri method.

CL registration was carried out in quantum-metric device, which was assembled by using a new developed electronic and amplifier unit. As a detector of weak light fluxes a highly sensitive low-noise photoelectronic multiplier PEM-140 (spectral sensitivity diapason 300–700 *nm*) was used.

Reliable chemiluminescent analysis was carried out by computer system of automatic registration and mathematical treatment of the obtained experimental data through USB 6008 data gathering device and program providing treated in LabVIEW medium. Device calibration was realized by etalon source of light of known intensity SPCM-1 N46 with absolute light flux $8.43 \cdot 10^5$ quantum/$s \cdot 4\pi$ [16].

3 *mL* of the sample was heated in a thermostat (37°*C*) and placed in cuvette. Control cuvette was placed against photocatode PEM, which provides high efficiency of registration. Radiation registration was started via 1–2 *s* after sample insertion. GR reagents and distilled cool-prepared water were used (pH 6.2).

For registration of the sample radiation, immediately before each measurement the determination of background noise of device was carried out. CL of the samples was recorded during 120 *s*. CL intensity was measured in conditional units (c.u.) and by radiation light-sum. Chemiluminescence curves were presented in Fig. 1.

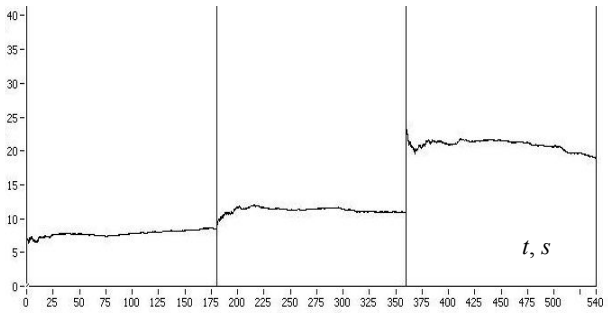

Fig. 1. Spontaneous CL of 3-day wheat seedlings:
1 – dark background of device;
2 – control;
3 – irradiated samples by EMI with 50.3 *GHz*, 1 *h*.

mean	sigma
7.795	0.4403
11.240	0.3942
20.890	0.7338

Accumulation of products of lipid peroxidation (MDA) was evaluated by color reaction with 2-thiobarbic acid, accepting that extinction molar coefficient of dyed complex with MDA is equal to $1.56 \cdot 10^5$ $M^{-1}cm^{-1}$ [17].

Results and Discussion. Results of CL analysis showed that the intensity of spontaneous CL of control herb roots and seedlings homogenate was approximately similar and equal to 11.79±0.47 c.u. and 14.56±0.43 c.u. respectively, when background noise of device was 7.90±0.85 c.u.

Study of EMI EHF effect on lipids peroxidation processes in plant organism showed that millimeter waves induce an oxidative stress not only in herb seedlings but also in roots depending on EMI frequency (Fig. 1).

Irradiation by 42.2 *GHz* frequency (wavelength 7.1 *mm*) does not invoke reliable changes of spontaneous CL intensity of homogenate of wheat seedlings and roots (Fig. 2), in the case, when EMI effects by 50.3 *GHz* frequency, leads to increasing of spontaneous CL intensity and consequently to light-sum of wheat seedlings and roots as compared with control by 38.4 and 30.5% respectively (Fig. 2). It should be mentioned that EMI by 51.8 *GHz* frequency makes the highest effect on intensity of free-radical processes enhancing the light-sum of spontaneous CL of seedlings by 1.41 times (42%) and roots by 1.31 times (31%) as compared with respective control.

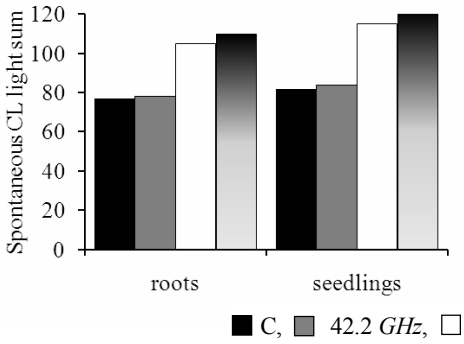

Fig. 2. Spontaneous CL light sum of 3-day wheat seedlings, irradiated by different EMI frequencies.

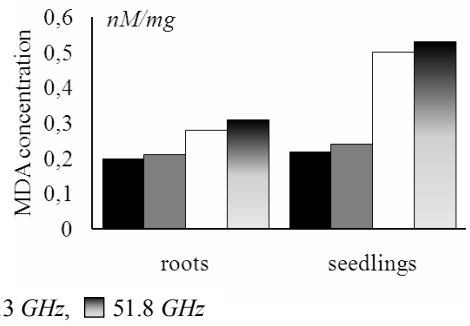

Fig. 3. MDA concentration in roots and seedlings of wheat, depending on EMI frequency.

The highest efficiency of EMI effect by 50.3 and 51.8 *GHz* frequencies on the intensity of free-radical processes, probably, can be explained by the fact that these frequencies correspond to own resonant frequencies of water molecule oscillations [1, 3, 11] composing 75–80% of the cell mass.

Earlier it was shown that EMI by 41.8, 42.2, 50.3 and 51.8 *GHz* frequencies, when it can be applied in short-time every-day exposition by 20 *min* during 5 days, is able to significantly stimulate MDA accumulation and activity of anti-oxidant enzymes of peroxidase and catalase in 3-day, 6-day and 9-day seedlings of wheat [15].

From the results presented in Fig. 3 it is obvious that MDA content in 4-day control seedlings and roots is almost similar and equal to 0.24±0.04 *nM/mg* protein and 0.20±0.03 *nM/mg* protein respectively. According to the obtained data EMI enhances MDA content (Fig. 3). At 1 *h* exposition of EMI radiation by 42.2 *GHz* frequency MDA concentration does not change, but in seedlings enhances by 12% as compared with control. EMI by 50.3 and 51.8 *GHz* frequencies has the highest effect on MDA growth. At irradiation by 50.3 *GHz* frequency MDA content in roots reliably ($p<0.01$) enhances by 1.40 times, in seedlings by 2.1 times compared with control. EMI by 51.8 *GHz* frequency has the highest effect on MDA formation. Thus, in roots MDA concentration is equal to 0.32±0.06 *nM/mg* protein exceeding that of control by 1.6 times; in seedlings 0.52±0.08 *nM/mg* protein exceeding that of control (0.24±0.04) by 2.08 times.

In the literature there is an analysis of possible cellular biochemical and physiological processes joined with EMI EHF effect on intracellular regulation and transduction of signals [1, 3, 6, 7]. The obtained data indicate that EMI EHF effects depending on irradiation frequency and induce free-radical oxidation processes in plant organisms which are registered by CL intensity and MDA accumulation.

Conclusion. The obtained data made it possible to assume that EMI EHF affects on oxidation of lipids of cellular membranes of herb seedlings and roots and lead to increasing the intensity of free-radical processes. It indicates that biological system is extremely sensitive to external effect of EMI, particularly by 50.3 and 51.8 *GHz*, which correspond to own resonant frequencies of water molecule oscillations.

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