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EFFECT OF EXTREMELY HIGH FREQUENCY EMI ON LIPID PEROXIDATION AND ACTIVIETES OF ANTIOXIDANT ENZYMES OF WHEAT SHOOTS

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The investigation of the electromagnetic irradiation (EMI) with extremely high frequencies (EHF)- 41.8 GHz, 42.2 GHz, 50.3 GHz and 51.8 GHz and low intensity flux capacity (0.6 $mW.cm^{-2}$) of seeds and growth of germs of wheat (*Triticum aestivum* L.) has been carried out. The effect of EMI on lipid peroxidation and antioxidant system function of wheat shoot cells has been also studied. It has been shown that at irradiation of germinating seeds the increasing of wheat germ mass as well as lipid peroxidation rate is observed. At the same time EMI-induced oxidative stress was indicated by the markedly change of catalase (CAT) activity depending on EMI frequency used and exposure duration.

Extremely high-frequency electromagnetic irradiation – Triticum aestivum L. – wheat germ shoot mass – catalase activity

Ուսումնասիրվել է ծայրահեղ բարձր հաճախականության (ԾԲՀ)- 41.8 ԳՀց, 42.2 ԳՀց, 50.3 ԳՀց և 51.8 ԳՀց և ցածր հոսքի խտությամբ՝ 0.6 մՎտ.սմ$^{-2}$ էլեկտրամագնիսական ճառագայթման (ԷՄՃ) ազդեցությունը ցորենի (*Triticum aestivum* L.) ծիլերի աճի վրա: Հետազոտվել է նաև ԷՄՃ ազդեցությունը ցորենի ծիլերի բջիջներում լիպիդների պերօքսիդային օքսիդացման ինտեն-սիվության և հակաօքսիդանտային համակարգի ֆունկցիաների վրա: Ցույց է տրվել, որ ծլող սերմերի ճառագայթահարումը բերում է ծիլերի զանգվածի, դրանցում լիպիդների պերօքսիդային օքսիդացման ինտենսիվության աճի: ԷՄՃ-ինդուկցված օքսիդատիվ սթրեսը արտահայտվում է նաև կատալազի ակտիվության նշանակալի փոփոխությամբ՝ կախված կիրառված հաճախականու-թյունից և ճառագայթահարման տևողությունից:

Ծայրահեղ բարձր հաճախականության էլեկտրամագնիսական ճառագայթում – Triticum aestivum L. – ցորենի ծիլերի զանգված – կատալազի ակտիվություն

Исследовалось влияние ЭМИ КВЧ-диапазона (41.8 ГГц, 42.2 ГГц, 50.3 ГГц и 51.8 ГГц) с плотностью потока мощности 0.6 мВт.см$^{-2}$ на рост семян пшеницы (*Triticum aestivum* L.*)*. Изучалось также воздействие ЭМИ на интенсивность процессов перекисного окисления липидов и функционирование антиоксидантной системы клеток проростков пшеницы. Установлено, что облучение прорастающих семян приводит к увеличению массы проростков и к усилению перекисного окисления липидов. При этом индуцированный ЭМИ оксидативный стресс приводил к определенному изменению активности каталазы в зависимости от частоты и длительности ЭМИ-воздействия.

ЭМИ КВЧ-диапазона – Triticum aestivum L. – вес проростков –активность каталазы

Nowadays the increasing number of artificial sources, structures and appliances that emit in EMI range of the electromagnetic spectrum, as well as using the radio-frequency part of spectrum, make the investigation of EHF irradiation influence on living matter very important.

The majority of researchers have shown that all membranes of different objects serve as the main location of influence for radiation in mm range: primary mechanisms which determine final effect of radiation in mm range influence are developed in the membranes [see for review [1] and references therein and [10]. It has been shown that EMI leads to changes of membrane properties: to acceleration or suppression in active ions transport, to changes in biological membranes permeability due to proteins conformation changes and by means of membrane lipid peroxidation [4-6, 9, 13].

Although a lot of reports regarding influence of EMI EHF are available, to our knowledge the mechanisms of induction of biological effects of high plants are not clear yet. Keeping in view the importance of wheat as an valuable yield crop and MM-waves as an environmental factor, the present study was designed to test effects of different EMI frequencies on growth (mass), lipid peroxidation and the activity of catalase in growing wheat shoots.

Materials and methods. *Plant Culture and Electromagnetic Irradiation Procedure:* The seeds of winter wheat **(***Triticum aestivum L.*) of "Bezostaya" variety were used in experiments. The germination of seeds was released in thermostat at 25^0C on wet filter paper in Petri dishes asit is described in [8]. The non-irradiated germinating seeds and seedlings were taken as control samples. Experimental samples- 2-, 3- and 4-day-old seedlings were irradiated every day 20 min with coherent electromagnetic waves of 41.8, 42.2, 50.3 and 51.8 GHz frequencies using a generator G4-141 type with working intervale of 37.50-53.57 GHz (State Scientific-Production Enterprise "Istok", Russia) and power flux density 0.6 $mW \cdot cm^{-2}$.

*Plant Extract preparation***:** The procedure of the plant extract preparation can be found in [8]. The supernatant of shoots homogenate was used for assessing the protein content by [11] and CAT activity using UV-visible Spectrophotometer (model SF-46, USSR).

*Analyses of lipid peroxidation***:** Lipid peroxidation in shoots was determined by estimation of the MDA content following the method of Costa H. [8] with slight modification [12].

*Catalase activity assays***:** Catalase CAT (EC 1.11.1.6) activity was measured by the method of Korolyuk M. [3], which is based on the reaction of the H_2O_2 in a mixture with ammonium molibdate ($(NH_3)_2 .MoO_4$).

Results and Discussion. *EMI effects on the growth of wheat plants***:** Effects of EHF EMI on growth wheat's shoots were carried out of 2-, 3- and 4-day-old plants. Growth intensity defined by mass of beforehand irradiated seeds shoots showed significant differences as compared with control, especially at the higher frequencies of EMI.

In case of irradiation with 42.2 GHz frequency the mass of germ shoots increases by 30% in the 3-day-old plants after seeds irradiation compared with the 2-day-old plants and by 74% in the fourth day after irradiation compared with the third day. At irradiation with 50.3 GHz these parameters were 30% and 74% and at irradiation with 51.8 GHz- 38% and 91% respectively. Besides, the activation of seedlings growth intensity showed the most magnitude value in 4-day-old plants at 50.3 GHz and 51.8 GHz frequencies, which are the water resonant frequencies as it is known well [1]. These and earlier data [14] coincided well.

*EMI effects on wheat seedlings cell lipid peroxidation content***:** It has been revealed that multiple irradiation of germinating seedlings of control seeds induced lipid peroxidation activity increase in germ cells the change magnitude of which depends on irradiation frequency and duration (fig. 1).

Obtained data showed, that EMI results by increase lipid peroxidation process activity, which expressed by MDA rate increasing. So, in case of wheat seedlings' irradiation by 41.8 GHz and 42.2 GHz frequencies MDA concentration showed trend for

increasing for each EMI duration according to the age of the shoots. Thus, in cells of 2- and 3-day-old shoots exposed to EMI by 41.8 GHz frequency the MDA amount increases by 44% and 55% for EMI 60 min summary duration and by 55% and 50% for 80 min duration, correspondingly as compared to control plants. At the same time, in shoots of 4-day-old experimental plants increasing of MDA rate was not observed, which witnessed that lipid peroxidation intensity does not exceed the appropriate control (fig. 1a). Approximately the same phenomenon were observed for 2- and 3-day-old wheat seedlings multiple irradiation with 42.2 GHz frequency (fig. 1b). But, for 4-day-old shoots MDA rate increased by 1.52- and 1.68-fold respectively for 60 min and 80 min EMI duration as compared with control. As it is obvious from fig. 1 (fig. 1, c, d) the greater responses of lipid peroxidation were recognized in case of wheat seedlings EMI by 50.3 GHz and 51.8 GHz frequencies. According to obtained data MDA content significantly ($P < 0.01$) increased in all the expose EMI and this increase reached its maximum at 60 min compared to control. On the other hand, the further irradiation for 80 min results by significant decline of MDA rate in all three ages experimental seedlings studied (fig. 1c, d).

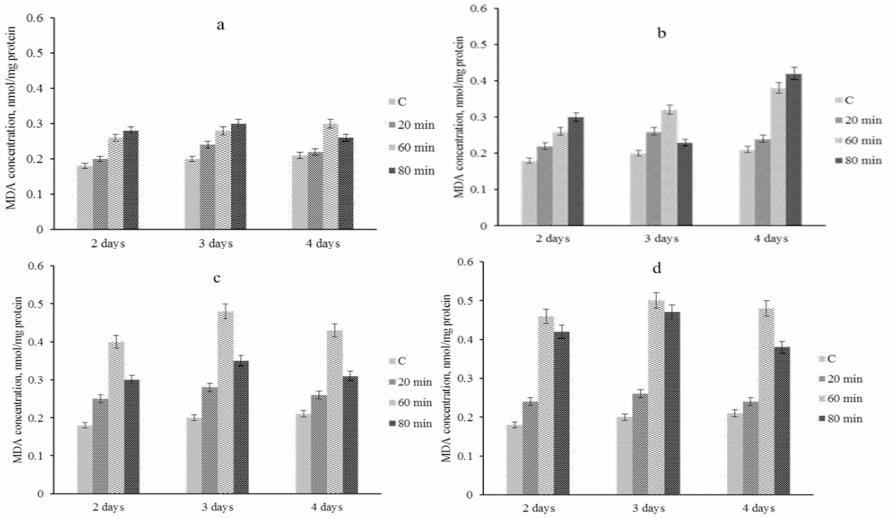

Fig. 1. The dependence of MDA rate in wheat seedlings during their growth on EMI duration with 41.8 GHz (a), 42.2 GHz (b), 50.3 GHz (c) and 51.8 GHz (d) frequencies.

Influence of EMI on catalase activity: It has been revealed that the irradiation of germinating seedlings induces catalase activity increase in them the change magnitude of which depends on EMI frequency and expose duration, as well as on plants age (fig. 2).

From the data represented in fig. 2, it is obvious that in case of irradiation with 41.8 GHz frequency catalase activity noticeable decrease was observed in the 3- and 4-day-old shoots, moreover this fact depends on irradiation duration. Interestingly, in case of EMI with 20 min duration in above mentioned age plants the catalase activity decreased by 13.4% and 6.4% and for 60 min irradiation- by 23.4 % and 13.1 % respectively. The further increase of irradiation duration up to 80 min causes a significant activation of catalase: more than 1.5-times compared to the 60 min duration EMI and by 13% compared to control 3-day-old plants. For the 4-day-old plants the same criteria were 1.33-times and 18% correspondingly. As it is obvious from represented figure (fig. 2c, d) the same regularity was observed during growth of irradiated shoots with 50.3 GHz and 51.8 GHz frequencies, moreover in case of last two EMI frequencies response was grater.

As it is obvious from represented figure (fig. 2, c, d) the same regularity was observed during growth of irradiated shoots with 50.3 GHz and 51.8 GHz frequencies, moreover in case of last two EMI frequencies response was grater.

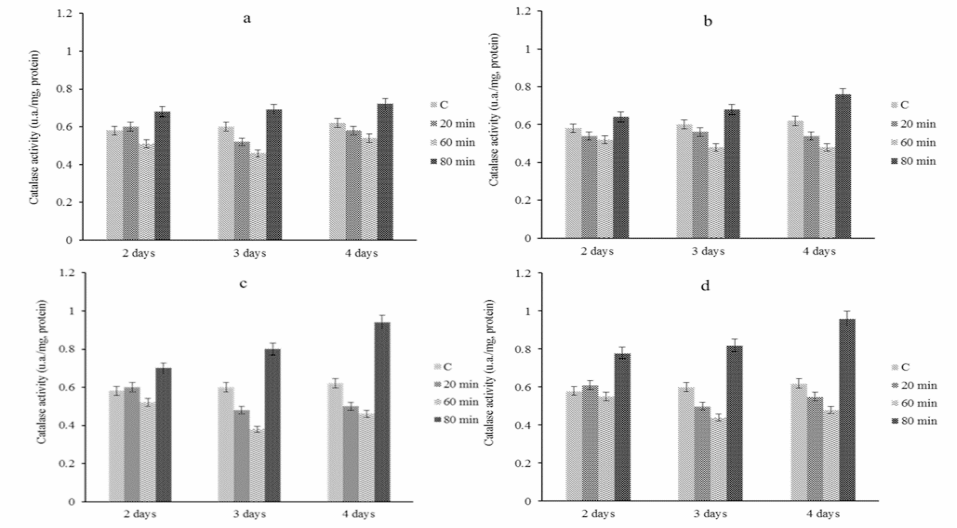

Fig. 2. The dependence of catalase activity in wheat shoots during their growth on EMI duration with 41.8 GHz (a), 42.2 GHz (b), 50.3 GHz (c) and 51.8 GHz (d) frequencies.

So, based on obtained data it is possible to conclude: in all age-plants exposed to EMI decrease of catalase activity is registered for 20- and 60 min exposure duration. Besides, parallel to the increase of EMI duration up to 80 min, enzyme activity increases in tested wheat shoots of the same age. At the same time, the catalase activity showed trend for increasing for each EMI duration, according to the age of the plants. The increasing activity of catalase appeared to be involved in effective scavenging ROS generated by EMI treatments.

The results of our study revealed the stimulation effects of EMI on wheat plants growth and the role of antioxidant defense system in organism's response to external physical field. According to obtained data one-fold EMI of wheat presoaked seeds induces increased shoot mass in all-age studied seedlings, but in the course of time (3-4-day-old shoots) the growth intensity was increased, especially at EMI 50.3 GHz and 51.8 GHz frequencies. This fact indicates that the biological effect of MM EMI on organism level is performed mainly at water resonant frequencies that show a certain role of water in biosystem response reaction [1, 7]. Increased shoots length and weight in winter wheat (*Triticum aestivum* L.), barley (*Horderum vulgare* L.) and maize (*Zea mays* L.) plants subjected to EMI have been reported [2, 14]. The results of present study are in a good conformity with these data [2, 14].

Obtained data indicated that there was a significant ($p < 0.04$) increase in MDA concentration of EMI exposed plants cell, and this increment was more pronounced at 50.3 GHz and 51.8 GHz, compared to control. In this concern, authors [3, 13] stated that the use of EMI at 42.8 GHz and 50.3 GHz frequencies leads to an accumulation of free radicals such as $O_2^{-\cdot}$ and H_2O_2 which unbalance the system of free radical elimination and thus induce MDA level. Increase in MDA content and antioxidant enzyme- catalase activity under EMI –stress in present study is consistent with the studies [2, 4, 6], in which wheat, barley and maize seeds were grown under EMI and TBAS values and antioxidative enzymes activities were found to increase.

Compering obtained results of MDA level and catalase activity in irradiated plant shoots we can notice that the comparatively increasing rate of MDA parallel to the duration increase for each applied EMI frequency is correlated to the suppression of catalase activity in the shoots of the same age. In the case of 80 min EMI multiple expose data about the sharp increase of catalase activity correlated well to slowdown of MDA level in the shoots of the same age. In plants submitted to external physical field SOD, CAT and GPX act as a defence mechanism which gets activated. The results obtained in current study also demonstrate that catalase protects wheat plant cells from the destructive effects of ROS and constitute key component of the cellular antioxidant defense system.

In conclusion our study showed that increases in mass, lipid peroxidation and catalase activities in *Triticum Aestivum L*. seedlings could been attributed to EHF EMI stress. MDA level change at plants irradiation indicates external physical field's effect on cell membrane properties. The grater response of plant organism was observed at water resonant frequencies which confirms that the primary element of EMI is water. In addition, the results may also suggest that increasing the activity of catalase plays an important role in ROS scavenging process and protect wheat plans against stress.

REFERENCES

1. *Девятков Н.Д., Голант М.Б., Бецкий О.В.* Миллиметровые волны и их роль в процессах жизнедеятельности. М., Радио и связи, 169 с, 1991.
2. *Калье М.И.*, Влияние КВЧ-излучения миллиметрового диапазона на физиологические процессы прорастание семян пивоваренного ячменя. Автореферат, Новогород: 119 с, 2011.
3. *Королюк М.А., Иванова Л.И., Майорова И.Г., Токарев В.Е.* Метод определения активности каталазы. Лаб дело, 1, 16-19, 1988.
4. *Мартынюк В.С., Темурьянц Н.А.* Роль перекисного окисления липидов и тиол-дисульфидного обмена в механизмах антистрессорного действия электромагнитного излучения крайне высокой частоты. Миллиметровые волны в биологии и медицине, 5, 61-69, 1995.
5. *Петров И.Ю., Бецкий О.В.* Изменение потенциалов плазматических мембран клеток листа зеленого растения при электромагнитном облучении. ДАН СССР, *305*, 2, 474-476, 1989.
6. *Шаров В.С., Казаринов К.Д., Андреев В.Е., Путвинский А.В., Бецкий О.В.* Ускорение перекисного окисления липидов под действием электромагнитного излучения миллиметрового диапазона. Биофизика, *28*, 423-427, 1983.
7. *Belyaev I.* Non-thermal biological effects of mico-waves: Current knowledge, further perspective, and urgent needs, Electromagnetic Biology and Medicine, *24*, 375-403, 2005.
8. *Costa H., Gallego S.M., Tomaro M.L.* Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons, Plant Science, *162*, 6, 939-945, 2002.
9. *Gill S., Tuteja N.* Reactive oxygen species and antioxidant mechinary in abiotic stress tolerance in crop plants, Plant Physiol. Biochem., *48*, 909-930, 2010.
10. *Guofen Yu., Coln E., Schoenbach K. Gellerman M., Fox P.,Rec., L. et al.* A study on biological effects of low-intensity millimeter waves, IEEE Transactions on Plasma Science, 30, 1489-1496, 2002.
11. *Lowry O.H., Reserbrough N.J., Farr S.L., Rondall R.L.* Protein measurement with Folin phenol reagent, J. Biol. Chem., *193*, 265-75, 1951.
12. *Poghosyan G.H., Mukhaelyan Zh.H., Vardevanyan P.H.* Influence of cadmium ions on growth and antioxidant system activity of wheat (*Triticum Aestivum* L.) Seedlings, International Journal of Scientific Research in Environmental Sciences, *10,* 2, 371-378, 2014.

13. *Torgomyan H., Trchounian A. Escherichia coli* MembraneAssociated Energy-dependent processes and sensitivity toward Antibiotics changes as responses to Low-intensity electromagnetic irradation of 70.6 and 73 GHz frequencies, Cell BiochemBiophys, *62*, 451-461, 2012.
14. Vardevanyan P., Nerkararyan A., Shahinyan M. Influence of low intensity coherent electromagnetic millimeter waves on growth and peroxidase total activity of wheat germs, J. of Exp. Biol. And Agricult. Sci., *1,* 1, 39-44, 2013.

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