INFLUENCE OF LOW INTENSITY COHERENT ELECTROMAGNETIC MILLIMETER WAVES ON GROWTH AND PEROXIDASE TOTAL ACTIVITY OF WHEAT GERMS

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ABSTRACT

The investigation of the effect of electromagnetic irradiation with extremely high frequencies (EMI EHF) and low intensity on germination of seeds and growth of germs of wheat has been carried out. The change of peroxidase total activity in wheat germ cells has been determined under the effect of mentioned external factor. The role of water in formation of biosystem reaction was also discussed in present work. It has been shown that at irradiation of germinating seeds the increasing of wheat germ shoot mass and peroxidase total activity is observed. The mentioned effects are expressed more obviously in case of moistening of dry seeds and cultivation of germinating seeds and germs by irradiated water which indicates that the primary target of this effect is water.

KEYWORDS
EMI EHF
Irradiated water
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Germ shoot mass
Peroxidase total activity

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1. Introduction

Nowadays the intensity of electromagnetic irradiation (EMI) has been rising in environment. Being an invisible factor of external environment, however EMI is not indifferent for living organisms. Recently obtained data have indicated that millimeter electromagnetic waves (MM EMW) with low intensity effect on both unicellular and multi cellular organisms. According to literature data the effect of millimeter waves results in increasing of immune resistance, changing of both activity of enzymatic reactions and growth rate of organisms, inhibiting of microorganism growth (Kalantaryan et al., 2011; Gapeyev and Chemeris, 2000; Tadevosyan et al., 2008). The effect is expressed on living material being on any level of organization - not only on organism, organ and cellular but also molecular levels, may effect on genome particularly there are data indicating that the mentioned factor influences on DNA thermo stability (Kalantaryan et al., 2011; Kalantaryan et al., 2010; Vardevanyan et al., 2012).

It is suggested that the influence of MM EMW differs on different levels of organisms. From this point of view it is interesting to investigate the effect of EMI on different levels of organization of living organisms. It has been shown that EMW of different wavelength interact with biological systems that are on different organization level (Kalantaryan et al., 2010; Babayan et al., 2006; Betskii et al., 2004; Devyatkov et al., 1991). The special attention is given to the problem of effect mechanisms of EMI with low intensity and extremely high frequencies (EHF) on living organisms that cannot be conditioned by thermal effects (Devyatkov et al., 1991; Betskii, 1992; Kurik, 2001). Permeability of rays of given diapason is very small moreover their effect on biosystem is significant, has peculiarities and depends on irradiation frequency (Nerkararyan et al., 2005; Smolyanskaya et al., 1979).

In this work the effect of EMI EHF with low intensity on growth as well as peroxidase total activity changes in wheat germinating seeds has been investigated to reveal the pre-crop treatment opportunity of seeds for plant growth stimulating by electromagnetic waves. Particularly the mass change of irradiated germinating seed shoots as well as germ shoots that were obtained from seeds moistened with irradiated water has been investigated during their growth. The participation of water in organism response reaction formation to external physical field effect has been discussed.

2. Materials and methods

The wheat (Triticum aestivum) seeds of “Bezostaya” sort were used in experiments. Two approaches were applied: in the first case the early germinated seeds were irradiated – the dry seeds had been moistened by non irradiated water during 12 hours, and then these seeds were germinated and irradiated during germination one-fold, moreover they were cultivated by non irradiated water every day. In the second case the seeds were moistened by previously irradiated water and they were cultivated by irradiated water of respective frequency and duration every day during their germination. The non irradiated germinating seeds were taken as control samples. The mass of these germinating seed shoots were determined. The germination of seeds was realized in thermostat in 25°C. The irradiation was performed using the generators G4-141 with working interval of 37.50-53.57GHz and power flux density 60μW/cm² and G4-142 with working interval of 53.57-78.33GHz and power flux density 50μW/cm². Frequency signal stability was ±0.05% and frequency deviation of output signal in persistent regime of generation did not exceed 6MHz. The irradiation was performed at following frequencies: 49GHz, 50.3GHz; 51.8GHz, 53GHz and 64.5GHz; with 20 min duration. Choose of applied frequencies was conditioned by the fact that water resonant and near them frequencies are in this interval. The plant extract was obtained in 0.15 M tris-HCl buffer that contains 0.1M DTT (1,4-dithiothreitol), pH 8 (0.2ml buffer was added per 100mg plant extract (the shoots of germs were grinded in cold pounder). The extraction was carried out on magnetic mixture during 30 minute in refrigerator. The obtained mass was centrifuged during 15 min with 18000g. The supernatant liquid was used in experiments. All procedures were performed in cold conditions. Peroxidase activity was determined taking into account the optic density change of reaction mixture during 2 min, at room temperature and 450nm wavelength by SF-4A spectrophotometer. The reaction in reaction mixture starts from H₂O₂ injection moment. Peroxidase catalyzes the pirogallol oxidation reaction by hydrogen peroxide (Baden and Corbett, 1979). The reaction mixture contains 1.1ml - H₂O₂, 0.8ml - 0.06M Na-P (natrium-phosphate) buffer (pH 6.8), 0.5ml – 0.003M pirogallol, 0.2ml – extract, 0.2ml – 0.15% H₂O₂. 0.2ml distilled water was added to control solution instead of extract. The solution of H₂O₂ was added directly before the measurement. Protein amount was determined by Lowry method (Lowry et al., 1951). Peroxidase total activity was calculated by formula

\[
A = \frac{\Delta D \cdot f}{c \cdot t}
\]

where A is the total activity of enzyme (optic density/min. per 1 mg of protein), \(\Delta D\) – optic density change, c – protein amount (mg/ml), f – dilution coefficient, t – duration of optic density measurement (min). The statistic treatment of obtained data was performed.

3. Results

In present work the influence of MM EMW with low intensity and different frequencies on wheat germ growth as well as the response reaction formation of biosystem depended on mentioned factor have been investigated. Biosystem response
reaction to external physical field effect was determined based on peroxidase total activity changes in germ cells.

It has been shown that MM EMW irradiation with 20 min duration effects on mass change of germinated seed shoots during their growth – the growth intensity change during germ development is different in control and irradiated samples. In the first day after irradiation germ growth intensity is stimulated but during the further days it decreases remaining higher from the growth intensity of control germs. Thus in control samples the mass of wheat germ shoots increases in the third day compared with the second day by 146.9% and in the fourth day by 40.2% compared with the third day. The growth intensity is changed otherwise in irradiated germs.

Thus in case of irradiation with 49GHz frequency the mass of germ shoots increases by 133.3% in the third day after irradiation compared with the second day and by 46.8% in the fourth day after irradiation compared with the third day. In case of irradiation with 50.3GHz these criteria were 119% and 54.9% respectively, at irradiation with 51.8GHz – 130.1% and 51.2%, at irradiation with 53GHz – 104.3% and 42%, at irradiation with 64.5GHz – 118.3% and 47% respectively. The studies showed that after irradiation in the fourth day the mass of germinating seed shoots increases by 14.7%, 12.9% and 9.1% compared with control ones at following frequencies of irradiation – 50.3GHz, 51.8GHz and 64.5GHz respectively. To reveal the primary target of EMI EHF the effect of irradiated water on growth of seeds has been also investigated. The results represented on figure 1 indicate that the growth intensity of the germs cultivated by irradiated water with respective frequency is higher than that of the germinated seeds that were irradiated one-fold during germination being cultivated by non irradiated water each day. Moreover the consequences of external physical field are preserved longer. The results also indicate that biosystem response depends on irradiation frequency.

As it is obvious from figure 1, the greater response is observed in case of cultivation of germinating seeds by irradiated water with water resonant frequencies – 50.3GHz, 51.8GHz and 64.5GHz. At irradiation with 50.3GHz frequency the mass of germ shoots in the third day of growth increases by 45.8% compared with the second day and in the fourth day by 77% compared with the third day. In case of cultivation with 51.8GHz these criteria were 76.6% and 79.9% respectively, for 53GHz frequency – 71.6% and 65.9% and for 64.5GHz frequency – 62.4% and 70.7%. In the fourth day the mass of shoots increases by 30.2%, 35.5% and 29.3% compared with the mass of control ones at following frequencies of irradiation – 50.3GHz, 51.8GHz and 64.5GHz respectively.

It has been revealed that the irradiation of germinating seeds induces peroxidase activity increase in germ cells the change magnitude of which depends on irradiation duration (figure 2).
During the growth the peroxidase activity is changed not only in control but also in irradiated germs of seeds, but the magnitude of change observing during growth is different. From the data represented on figure 2 it is obvious that in case of irradiation with 49GHz frequency peroxidase activity noticeable increase is observed in the second and the third days after irradiation, moreover this fact depends on irradiation duration. Particularly in case of irradiation with 20 min duration in the second and the third days the peroxidase total activity increases by 52.7% and 78.8% respectively compared with control samples. In case of irradiation with 30 min duration these criteria were 91.9% and 90% respectively. In the fourth day after irradiation the view is changed: at 20 min duration peroxidase activity is almost the same in experimental and control samples, at 30 min duration it continues to rise and is higher by 125% compared with control, at 60 min duration - 162%. As it is obvious from represented figure the same regularity is observed during the growth process of irradiated germs with 50.3GHz, 51.8GHz and 64.5GHz frequencies, moreover in case of last three frequencies the response is greater.

The same regularity is observed in case of moistening and further cultivation of germinating wheat seeds by irradiated water with the respective duration and frequencies figure 3. Obtained data indicate that germ cultivation by irradiated water induces greater changes of peroxidase total activity than one-fold irradiation of germinating seeds. In this case also the dependence of biological system response magnitude on irradiation duration is observed. Besides as it is obvious from figures 2 and 3 biosystem response induced by irradiation depends also on EMI frequency.

Peroxidase total activity change is greater at cultivation of germinating seeds by irradiated water with 50.3GHz, 51.8GHz and 64.5GHz frequencies.

3. Discussion and conclusion

Obtained data indicate that millimeter waves have high biological activity even at low energy flux density. Based on investigations different hypotheses have been suggested concerning EMI effect primary target (Betskii et al., 2004; Betskii, 1992; Gayev and Chemeris, 1999; Tambiev and Kritkova, 2000; Petrosyan et al., 2001; Devyatkov et al., 1981). According to one of these hypotheses the primary target of EMI is water.

Fesenko and Gluvstein (1995) reported that at irradiation of water with extremely high frequencies the cluster shifting takes place which results in deviation of water structure geometry and tense development in system. The water having such property is like polymer and the water as polymer structure has a relaxation long time which is not equal to $10^{11}-10^{12}$ sec., but min. and longer. Therefore the energy of EMI quantum turning to internal energy of water organized structure in result of structure deviation is accumulated before becoming equal to hydrogen bond energy which is 500-1000 times bigger compared with electromagnetic field energy. When the magnitude of accumulated energy reaches to that of hydrogen bond the break of hydrogen bonds takes place and the structure is destroyed. Free ions and hydrated electron are formed. Therefore as a result of EMI EHF effect on water cluster structure the energy accumulation occurs until the critical value after which the break of bonds is observed and the released energy may be transformed into other forms of energy. If such changes of water properties take place in biosystem, water will inevitably take participation to biosystem response formation to that effect (Kalantaryan et al., 2010; Babayan et al., 2006). Our results are in correspondence to these data. Particularly the irradiation stimulates the growth of wheat germs which is conditioned by metabolism intensity growth, simultaneously peroxidase enzyme activity increase indicates that oxidation reactions of metabolic processes are stimulated.

Thus obtained data indicate that in cases of irradiation with 49GHz and 53GHz frequencies the slower growth of germs is observed compared with the other three frequencies. It becomes obvious from results that the greater effect is observed in case of irradiation with 50.3GHz, 51.8GHz and 64.5GHz frequencies. This fact indicates that the biological effect of MM EMW on organism level is performed mainly at water resonant frequencies that show a certain role of water in biosystem response reaction formation process to external effect. As it is followed from obtained data the effect of irradiation on germinating seeds is expressed faster: in the second and the third days after irradiation the growth intensity is higher compared with that of germinating seeds moistened and cultivated by irradiated water. Moreover in case of germinating seeds moistened and cultivated by irradiated water the growth intensity is preserving longer, that also maintains the suggestion that external physical field effect on biosystem is mediated by water since the effect of irradiated water turns the biosystem reaction more obvious: the accumulative effect is observed at germs exposed to long time irradiation - biosystem response increases with the time: in the fourth day after irradiation in case of cultivation by irradiated water the growth intensity is the greatest compared with other days in contrary of the growth dynamics of germs exposed to one-fold irradiation when the growth intensity starts decreasing in the fourth day after irradiation.

The fact that at irradiation of germs as well as at germ cultivation by irradiated water the greater reaction is observed in variants that were exposed to irradiation with 50.3GHz, 51.8GHz and 64.5GHz frequencies indicates that water is really the primary target of the effect and irradiation energy is absorbed by resonant mechanism. EMW EHF irradiation with low intensity changes the water structure of surface layers which results in changing of water properties in these layers. The last fact may induce the change of physical-chemical properties of cellular membranes that in its turn induce a chain of sequential reactions and due to them the biosystem response to external physical field effect is formed. Since peroxidase total activity increases in germ extract it is possible that...
enzyme synthesis controlling genes are activated as a response of stimulation of oxidation processes under the physical field influence. It is also possible that the revealed changes are conditioned by EMW EHF direct effect on enzyme and activation of already synthesized enzyme. Such effect of external field may be realized by regulation of auto-oscillations of molecules. It is known that in the absence of external effect the biological structures including proteins and nucleic acids are in auto-oscillation regime (Afromeev et al., 1997). The external physical field may increase the amplitude of auto-oscillations that may result in structural changes of molecules.

Peroxidase has a quaternary structure and if external EMW effect is realized via regulation of auto-oscillations peroxidase activity change may be conditioned by dissociation or re-association of enzyme subunits as a result of which the isoenzyme composition may be changed that in its turn results in enzyme total activity changing in conditions of our experiment.

Therefore obtained data indicate that the primary element of EMI EHF effect on biosystem is water since the greater response of biosystem is observed at irradiation with water resonant frequencies. Irradiation of seeds and cultivation of seeds and its germs by irradiated water stimulate the growth of wheat germs as well as result in increasing of peroxidase activity in germ cells.

Figure 3. The dependence of peroxidase total activity (A) in wheat germ cells cultivated by irradiated water with 49GHz (a), 50.3GHz (b), 51.8GHz (c) and 64.5 GHz (d) frequencies on EMI duration.
References


