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Original article

Influence of low-frequency electromagnetic fields against the germination of wheat (*Triticum aestivum L.*) seeds

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Abstract

The biological effects produced by low-frequency of the electromagnetic fields on the seed germination of the wheat were studied on Triticum aestivum L. Electromagnetic fields with frequencies between 10 and 200 Hz and an increasing of the intensity up to 5 Hz have been used. The density value applied to the electromagnetic fields was 2mT. Wheat (*Triticum aestivum*) seeds were used as biological material. The biological response was established by determining seed germination and it was performed by measuring the length of the germinated seed seedlings hypocotyls. The peroxidase level has been used as biological marker for both seedling hypocotyls and rootlets. The biological response was very complex; depending on the frequency of the fields, the effect can be stimulating or inhibitory.

Keywords

low-frequency EMF, wheat, germination, hypocotyls, peroxidase.

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Introduction

The species *Triticum aestivum* L., common wheat is one of the parental species from the Graminaceae family, together with other species and the wheat genotypes; their seeds have a complex chemical structure and have been scientifically analyzed [1-6]. From the organoleptic studies, to the sensory indicators (the presence of pests), from the biochemical markers to the fungal indicators, etc., we have been shown that the seeds of this species (*Triticum aestivum* L.), are a true chemical ecosystem [4, 5, 7- 9].

From the chemical markers studied, in wheat seeds (*Triticum aestivum* L. etc.) we can mention some oxidoreductase enzymes (peroxidase, catalase, amylase, etc.,), with oxidative stress effect [10-17].

The biochemical researche at the level of the wheat seed have been varied. Some of the results shown the identification of chemical substances such as phosphorus, cobalt, cadmium, NaCl. etc., hydrates carbon, which can be essential or toxic, as mycotoxins, etc., [8, 18-23]. The researche on fungal indicators, with microbial analysis of the wheat seed have also shown the identification of fungal strains, such as *Fusarium graminearum, Puccinia recondita, Rhizopus* sp., *Alternaria* sp*., Aspergillus* sp*.*, which developed various diseases, some of which had been used in biotechnology [5, 20, 24 - 28].

The results of the research carried out on the *Triticum aestivum* L. have been numerous but they can be complemented with results from the application of low–frequency electromagnetic fields against the germination of wheat seeds [29, 30].

The explosive increasing presence of the electromagnetic fields (EMF) in everyday life imposes a systematic study of their biological effects, especially for some particular EMF, such as some extreme low frequencies, radio frequencies and static magnetic fields. Besides the well-known thermal effects, EMF present a large palette of non-thermal biological effects with complex and yet not well-known effects on living organisms [31-35].

As such, electromagnetic fields induce various biological responses, either in cereals [31, 36- 40] or in the other plant species [37, 39 - 42].

Microorganisms are also known to be influenced by EMF [29, 43 - 46], in all situations the biological responses depending on the frequencies used and the fields intensities. Regarding the possible mechanisms of the interaction between EMF and the living matter, various hypotheses have been proposed in order to satisfactorily explain the effects of EMF [44, 47, 48].

According to these models, besides the classical thermal effects, more non-thermal effects, are discussed including: free radical effects, resonance effects (connected to the concept of "biological windows") or coherence effects induced by EMF in the living matter [5, 44, 47, 48]. Other studies have shown that ELF-EMF may lead to the alteration of the ion channels permeability, especially Ca^{2+}, in the cell membrane [1, 34, 49].

The effect of some frequencies has been explained based of different activities of various ions (especially K^+) in the replacing calcium ions, by using ion-cyclotron resonance [49, 50]. The frequency that activates the "frequency window" corresponding to the ion-cyclotron resonance values, and their harmonics, are related to the biological involved ions (mainly potassium and calcium). The results show either the increase or decrease of the permeability of the membrane, depending on the involved ion, which, in turn, may lead to the stimulation or inhibition of the plant cells growth [51].

Other models used to explain the biological effects of ELF-EMF include the interaction between ferrymagnetic crystals (such as crystalline magnetite Fe_3O_4, ε-Fe_2O_3 and hematite α-Fe_2O_3) present in the plant cells and the EMF, interaction that leads to a torque under weak magnetic fields, which is strong enough to lead modifications of the membrane permeability in the case of ions such as potassium and calcium [34, 44, 52].

Other studies suggest that EMF induces changes in the structure of the water molecules, due to the quantum coherence [53], this type of molecules being one of the possible primary targets responsible for nonthermal biological effects of EMF. Other models such as the radical-pair mechanism have also been used [49, 53].

ELF-EMF may also have an effect on several cellular functions, such as cell proliferation and differentiation, the DNA synthesis, the RNA transcription, the ATP synthesis and the hormone production.

Previous studies have concluded that biological cells are bio-electrochemical structures which interact with their environment in various ways: physically, chemically, biochemically, and electrically.

This paper studies the biological effects on seed germination of wheat, produced by extremely low frequency (ELF), (i.e. in a frequency window ranging from 10 to 200 Hz) and a magnetic field of 2mT. The biological response was established by germination determinations, and by the measuring the length of the seeds germinated seedlings hypocotyls.

The germination test determines that germination potential within a series of seed samples can be used to compare the quality of different series and estimate the field planting values. The aim of these researche is to determine the biological response of the germinated seeds under various external stimuli conditions. Nevertheless, germination tests are very simple, little time- consuming, cheap and, therefore, could be ideal methods to test the influence of external factors, such as ELF-EMF. Thus, we selected this method due to its simplicity, speed and sensitivity [54,55].

Materials and Methods

The EMF has been obtained by a homemade system comporting a magnetic coil with a ferromagnetic core stimulated by an amplified sinusoidal that allows tuning its frequency. The germination tests were performed in a growth chamber Conviron MP4030, model G30, with programmed temperature, humidity and light.

Seed samples of wheat (*Triticum aestivum* L.), were obtained from S.C.D.A. Suceava, and their germination rate was measured.

Electromagnetic fields with frequencies varying between 10 and 200 Hz by steps of 5 Hz were used. The flux density value of the applied magnetic fields was 2mT and it was measured with a KOSHAVA 5 telemeter.

A series of tests have been conducted on three replicates of 100 seeds. Each series of seeds was characterized by dried weight (12%). Seeds were exposed to the electromagnetic fields in test tubes for 30 minutes, and sown on filter paper placed in Petri dishes using bi-distilled water in a growth chamber Conviron MP4030, model G30. An unexposed blank (3 x 100 seeds) batch was also used in these experiments. Seeds were maintained in the growth chamber at constant temperature and humidity regimes (20°C and 85%, respectively), in the dark, until an embryo elongation (hypocotyls and rootlets) was established. Seeds with visible coleorhizae were considered as germinated. The seeds were periodically moistened and the percent of germinated seeds (the germination rate, G_R) was reported after 7 days. Young wheat plants were subsequently harvested from their seeds and measured. The average length of hypocotyls, L_H, were expressed in cm.

The peroxidase level was measured on both hypocotyls and rootlets, whereas an oxidoreductase induces an important metabolic answer in the living organism under stress [53]. In our case the stress stimulus was the applied electromagnetic field at different frequencies. To this end, 5 mg of vegetal material (rootlets, hypocotyls) were grinded using quartz sand in an ice bath. The extract mixed with a buffer solution (pH 7) and the resulting homogenous mixture were centrifuged for 15 minutes at 3000 rotation/min. The supernatant selection represents the enzyme source. The peroxidase was identified with o - dianisidine and expressed as peroxidase units (PU), namely the amount of enzyme that decompose the hydrogen peroxide (UP/mg/min) [2,56].

The data were validated by the variance analysis and the ***t*** test [50,57].

Results and Discussions

Experiments have been conducted to determine the influence of the electromagnetic field on the germination of wheat seed (hypocotyls length, peroxidase level at hypocotyl and rootles). The results obtained from these experiments are presented in the Table 1 and Figures 1-3.

The results regarding the increase in length (L_H) of the wheat hypocotyls were statistically interpreted, expressed by the growth deviation between the samples and the refer-

Table 1. Average values of the hypocotyls length (L_H), in mm, of germinated wheat seeds as an effect of the electromagnetic fields at various frequencies

Frequency (Hz)	L_H (mm)	Difference (+/-)		Significance	Frequency (Hz)	L_H (mm)	Difference (+/-)		Significance
		mm	%				mm	%	
reference	75.1±1.83	-	-	-	105	70.5±2.02	-4.59	-6.11	0
10	78.0±2.01	2.94	3.91	-	110	39.4±1.44	-35.70	-47.54	000
15	63.9±1.66	-11.13	-14.83	00	115	79.5±2.39	4.39	5.85	*
20	71.3±2.17	-3.73	-4.97	0	120	70.3±1.95	-4.76	-6.34	0
25	53.2±1.44	-21.84	-29.08	000	125	77.5±2.44	2.46	3.28	-
30	76.5±1.58	1.47	1.96	-	130	78.6±2.75	3.56	4.74	*
35	70.9±1.55	-4.17	-5.55	0	135	94.7±3.04	19.61	26.11	***
40	108.2±2.88	33.12	44.21	***	140	81.9±2.62	6.85	9.12	*
45	64.5±1.62	-10.53	-14.02	00	145	84.0±3.05	8.94	11.90	**
50	74.6±1.84	-0.43	-0.57	-	150	77.0±2.59	1.98	2.64	-
55	76.2±1.91	1.11	1.48	-	155	60.1±2.29	-15.00	-19.97	000
60	72.0±1.83	-3.06	-4.07	-	160	83.4±1.88	8.37	11.15	**
65	71.4±2.32	-3.63	-4.83	0	165	77.4±1.83	2.32	3.09	-
70	60.4±2.00	-14.68	-19.55	000	170	69.6±2.01	-5.45	-7.26	0
75	72.7±1.79	-2.34	-3.12	-	175	86.4±2.11	11.37	15.14	**
80	74.5±2.69	-0.55	-0.73	-	180	86.0±2.24	10.96	14.59	**
85	77.0±2.53	1.98	2.64	-	185	65.4±1.68	-9.63	-12.82	00
90	74.3±2.06	-0.75	-1.00	-	190	95.3±2.22	20.31	27.04	***
95	72.9±1.67	-2.15	-2.86	-	195	86.9±2.54	11.85	15.78	**
100	75.1±1.91	0.04	0.05	-	200	91.1±2.87	16.00	21.30	***

(signify cance levels) : *and 0 (α = 0.10); ** and 00 (α = 0.05); *** and 000 (α = 0.01); - not significant; * positive and 0 negative deviations)

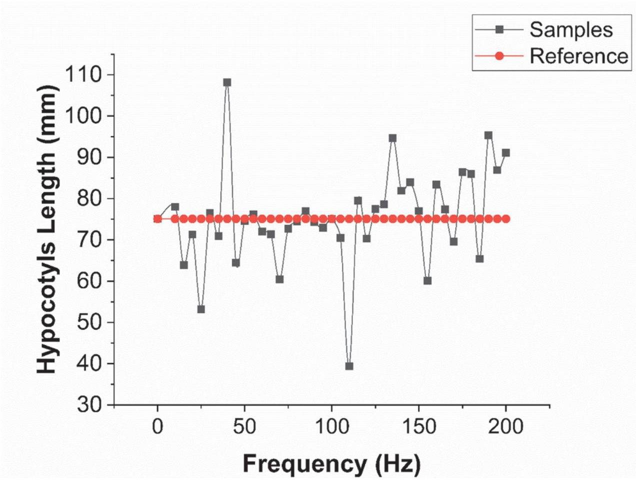

Fig. 1.

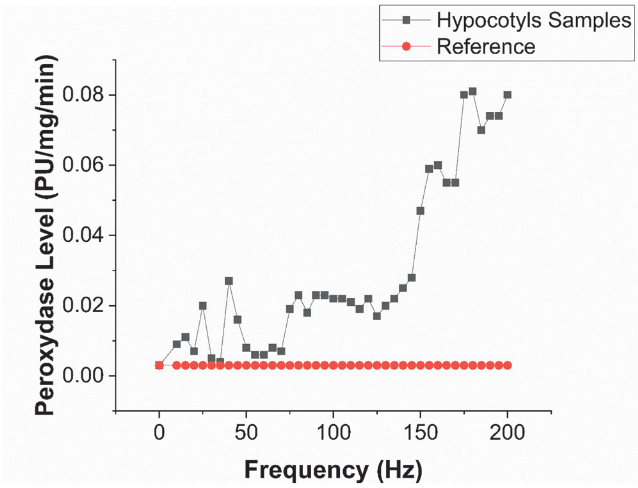

Fig. 2.

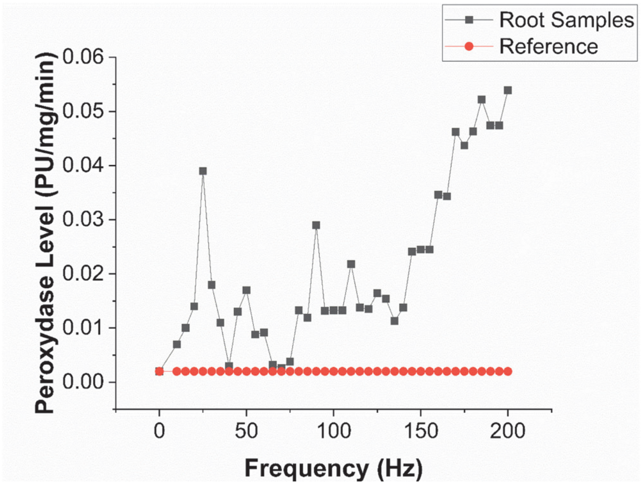

Fig. 3.

ence, under the influence of the frequency electromagnetic field. Positive growth deviations were obtained at a frequencies of 40, 135, 190, 200Hz; while negative growth deviations were recorder under the influence of frequencies of 45, 70, 110, 155, 175 Hz., for other frequencies the results were not significant (table 1).

A first remark is that the treatments influenced the hypocotyls growth only; the germination rates were not influenced significantly. On the one hand, a series of electromagnetic frequencies inflicts significant changes on the hypocotyls elongation in such a way that the biological response has a very complex pattern. Thus, frequencies of 40, 135, 190 and 200 Hz, induced substantial stimulation (over 20 %) of the hypocotyls growth, with the maximum value (44 %) observed for a frequency of 40 Hz. On the other hand, frequencies of 25, 70, 110 and 155 Hz induce very significant inhibitions; at 110 Hz the inhibition were almost 50% (figure 1).

The theoretical considerations presented above cannot completely account for the processes observed experimentally. This is because the seeds were electro-magnetically treated prior to germination, while in dormancy status whereby the embryo's metabolic processes were inhibited to a maximum level. The « water hypothesis» is also unsatisfactory because the water content of seeds was very low (12%) during the ELF-EMF exposure, and therefore, the EMF was unable to induce different (stimulations or inhibition of over 40 %!) in the germination process. On the other hand, the assumptions considered in this study, such as ion-cyclotron resonance or the radical-pair formation can only partly explain the stimulating or inhibiting events, or the absence of these events, as a function of the frequency of the applied ELF-EMF.

We hypothesize that the information transmitted only by certain frequencies, signal forms and intensities of ELF-EMF interfere with the wheat embryo biostructure, thereby inducing, through the coordination between levels, changes in the molecular structure observed experimentally in the above-mentioned studies.

Moreover, the amount of peroxidase of the samples studied was higher than that of the reference sample and this content increased with the frequency (Figures 2 and 3).

The process is similar to the disruption of the functioning of some electronic equipment by an external signal with certain parameters. In support of this claim it can be observed that the peroxidase levels increased, both in the germinated wheat hypocotyls, wheat rootlets. The peroxidase activity recorded different value under the influence of the entire frequency spectrum. At the wheat hypocotyls the peroxidase activity was stimulated at a frequency of 175 -200 Hz., with a value of 0, 08 PU/ mg/min., (figure 2).

At the wheat rootlets the peroxidase activity was stimulated by the same frequency electromagnetic field, with a value of 0, 06 PU/ mg/min. As frequencies and waveforms of the EMF signal used by us were conventionally selected (sinusoidal signal, beginning from 10 Hz with a 5 Hz increment); only those signals that are located in the sensible "area" of the bio-field created by the embryo biostructure could resonate with it, leading to the producing of the effects observed macroscopically (figure 3).

A finer modulation of EMF values could highlight the fine energetic structure of the studied plants. Further investigations are necessary in order to establish all the aspects of the intimate action mechanism of ELF-EMF against the seeds germination.

Our results are edifying and together with existing ones [5, 7, 8, 25, 47] they come to confirm that wheat seeds have a metabolic plasticity and remain on open path for the applying research ideas.

Conclusions

The effect of ELF-EMF on the germination and seedling growth of wheat seed were investigated. The length of hypocotyls varied as a function of the EMF frequency. The biological response, stimulation or inhibition of the hypocotyls growth was found to depend on the applied frequency of the EMF.

The biological response is considered as a result of the interference between the EMF and the field produced by the living bio - structured fields, in our case by the wheat embryos. The intensity of the biological response depends on the resonance level between the applied fields and the bio - structured field of the wheat seeds.

All of our research was statistically validated.

Conflict of interest

The authors declare that they have no conflicts of interest.

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