THE INFLUENCE OF EXTREMELY LOW FREQUENCY MAGNETIC FIELD ON TREE SEEDLINGS*

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This study is focused on the biochemical changes induced by extremely low frequency magnetic field in arbor seedlings (black locust, *i.e. Robinia pseudoacacia*) of ecological interest during their early ontogenetic stages. Chronic exposure has been done by daily exposures of 0.5–1–2–4–8 hours, to young *Robinia pseudoacacia* seedlings (two months old), during 10 days. Assimilatory pigments and average nucleic acid level were assayed after low frequency magnetic field exposure, by spectrophotometric methods. Certain stimulation of chlorophyll biosynthesis was noticed while inhibitory effect upon the nucleic acid biosynthesis was also revealed.

Key words: extremely low frequency magnetic field, *Robinia pseudoacacia*, photoassimilatory pigments, nucleic acids.

1. INTRODUCTION

This study was planed to reveal the possible physical basis for new biotechnological tool in conducting the development of fast growing tree species *Robinia pseudoacacia* considering its ecological importance in many geographic areas. The study was carried out in the more general context shaped by the fact that plants are becoming more and more suitable systems for the emphasizing of magnetic fields biological effects. Sensitivity of young plantlets to magnetic field continues to represent a challenging issue of bio-electromagnetism, the diversity of biological material, exposure systems and analysis methods enlarging continuously the frame of multidisciplinary research. Numerous earlier experiments proved that the static or sinusoidal magnetic fields had measurable effects on various living organisms. Effects of the magnetic field generated by alternating currents of 50/60 Hz and of the scattered electromagnetic fields of power line and electric engine were investigated on human and animal organisms [1–3], as

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well as the effect of scattered electromagnetic fields on the germination and growth of plants [4–7]. Low frequency magnetic field effects on plants are less studied, but may contribute to the understanding of the underlying basic mechanisms. Davies [8] was reported an increase in weight and size for plant radish under influence of magnetic field of 60 Hz and 40 μT. Frequency dependent changes in membrane permeability were reported for barley seeds by Khizhenkov [9]. The preliminary study presented in this paper was focused on the biochemical changes induced by extremely low frequency magnetic field in arbor seedlings (black locust, *i.e. Robinia pseudoacacia*) during their early ontogenetic stages.

2. MATERIALS AND METHODS

Black locust seedlings aged of 2 months developed from the tree pods harvested from the same adult tree of *Robinia pseudoacacia,* in order to diminish the putative genophond variations, were studied. The seedlings have been let to grow up in adequate recipients containing soil samples from the originally locust forest.

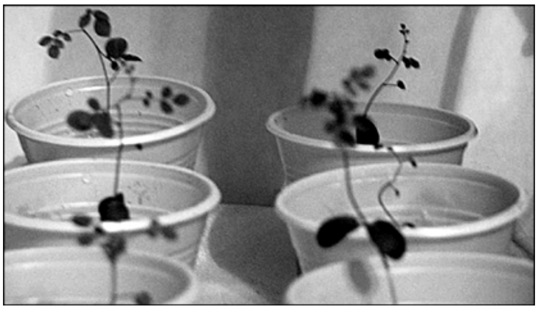
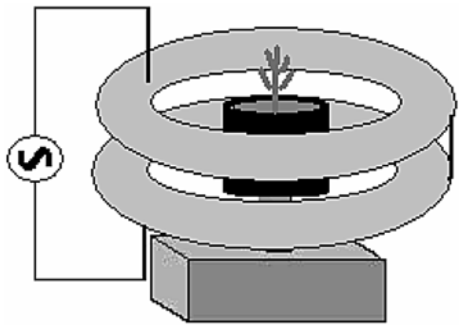


Fig. 1 – Experimental samples of *Robinia pseudoacacia* seedlings two months aged.

Black locust (*Robinia pseudoacacia*) seedlings aged 2 months have been grown in well controlled laboratory conditions of temperature (24 ± 0.5°C) and illumination (10 h: 14 h light/dark circle), in dishes containing adequate quantity of soil. All plant groups were supplied daily only with deionized water during the experiment, 6ml water per each recipient with seedling. The vertical sinusoidal magnetic field (50 Hz, 10 mT) was generated by means of a pair of Helmholtz coils system, able to generate a uniform magnetic field into a relatively large space volume allowing plant uniform exposure. Fig. 2 shows a schematic photo of the exposure device. Each Helmholtz coil from exposure device has a diameter by 260 mm and 1000 number of turns. The Helmholtz coils system is able to generate 10 mT, 50 Hz time-varying magnetic field. Chronic exposure has been done by daily exposures of 0.5–1–2–4–8 hours, to

Fig. 2 – Schematic image for magnetic exposure device.



young *Robinia pseudoacacia* seedlings during 10 days. During the magnetic exposure, the ambient temperature was the same for the control and the exposed samples (23÷0.5°C). Magnetic field induction in the experimental room, of about 0.4 ± 0.05 μT, was measured by means C.A.40 Gaussmeter. Magnetic treatment was provided as magnetic energy doses: $D = w \cdot t$, where w [J/m^3] is the energy density of magnetic field and t [s] is the exposure time duration. The magnetic field energy density is defined as: $w = \frac{B^2}{2\mu_0}$, where: B [T] is the magnetic field induction and μ_0 is the magnetic permeability of free space.

The magnetic field energy density available within this study was of 40 J/m^3. After 10 days of low frequency magnetic exposure spectrophotometric assays were accomplished: the content of chlorophyll a and chlorophyll b and total carotenoid pigments (Meyer-Berthenrath's method modified by Ştirban [10]) and the average content of nucleic acids (Spirin's method [11]). The spectral device was a Perkin-Elmer spectrophotometer UV-VIS provided with quartz cells. Biological material consisted of green tissue from seedling leaves, both for magnetically exposed samples and control. Assimilatory pigment contents were measured by spectrophotometrical assay upon extracts in acetone 85% [10], while the average nucleic acid level upon extracts in perchloric acid 6% [11]. The levels of chlorophyll a, chlorophyll b and total carotenoid pigments may be calculated with the next formulae according to [11]:

$$\text{chl a} = \frac{12{,}3 \cdot A(663) - 0{,}86 \cdot A(645)}{1000 \cdot d \cdot w} \cdot V,$$

$$\text{chl b} = \frac{19{,}3 \cdot A(645) - 3{,}6 \cdot A(663)}{1000 \cdot d \cdot w} \cdot V,$$

$$\text{t.c.} = \frac{10 \cdot A(472)}{2485 \cdot d \cdot w} \cdot V,$$

while the average content of DNA and RNA is given by the relation [15]:

$$[AN] = \frac{E(270) - E(290)}{0.19} \frac{V}{w}$$

where: w – the fresh vegetal sample mass, V – the extract volume; $A(\lambda)$ – the light absorption to the wavelength λ; d – the quartz cell width.

3. RESULTS AND DISCUSSIONS

The assimilatory pigment levels have been comparatively studied on the basis of graphical representations of chlorophyll a, chlorophyll b and total carotenoids. In Fig. 3 the stimulatory influence of extremely low frequency magnetic field exposures on the biosynthesis of all assimilatory pigments analyzed was revealed for low exposure times (0.5 and 1 hour). For enhanced exposure times an inhibitory effect was observed. The assimilatory pigments sum appear increased for the experimental samples exposed for 0.5 and 1 hour, while for longer exposure times a decreased level could be noticed.

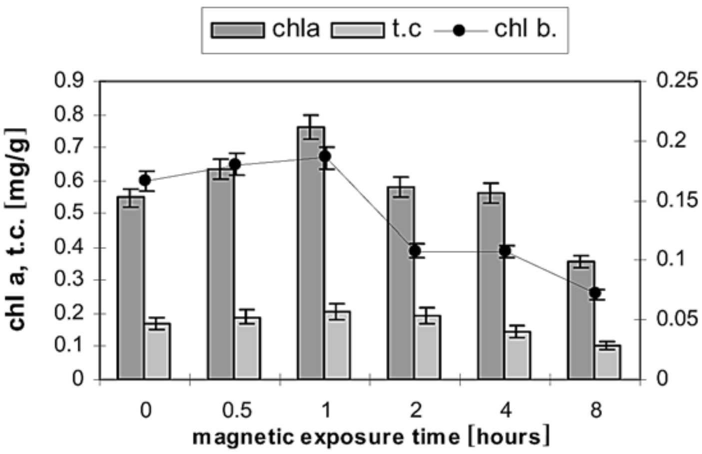


Fig. 3 – Assimilatory pigments level in young *Robinia pseudoacacia* seedlings (chl a – the content of chlorophyll a, chl b – the content of chlorophyll b, t.c. – the content of total carotenoid pigments).

In Fig. 4 is presented the assimilatory pigments sum versus the magnetic field dose used during experimental magneticexposure. The variation of total assimilatory pigments levels in young *Robinia pseudoacacia* seedlings under the extremely low magnetic field exposure is similar to chlorophyll a level.

Between chlorophyll **a** and total carotenoids levels a good linear correlation was established, chl a = 3.1898t.c. + 0.0371, with correlation coefficient $R = 0.9152$. These results can be taken as an indication upon the correlated influences of extremely low frequency magnetic field in both chlorophyll **a** and the secondary

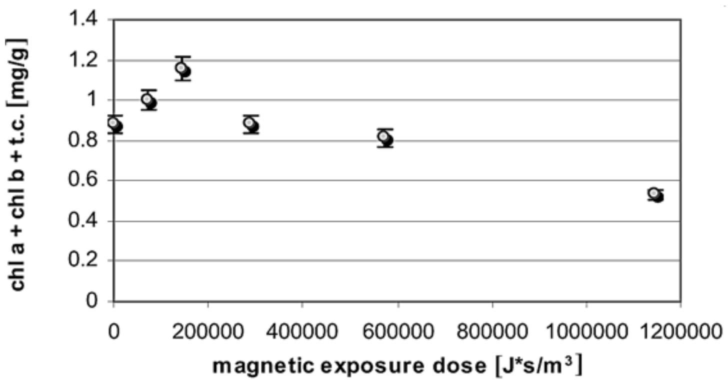


Fig. 4 – The assimilatory pigments sum in young *Robinia pseudoacacia* seedling.

carotenoids like pigments (with a very consistent role in the sustaining of photosynthesis efficiency, by means of the absorbed energy transfer to chlorophyll a molecules). The chlorophylls ratio is also providing important indirect data on the photosynthesis since it is an indicator on the activity of Light Harvesting Complex II (LHC II) from the vegetal cell tylakoidal structures (that is known as an enzyme complex rather sensitive to the action of external factors).

The chlorophylls ratio for extremely low frequency magnetic field exposure of young *Robinia pseudoacacia* seedlings is given in Fig. 5. This ratio being considered an important physiological parameter regarding the photosynthesis efficiency, the results displayed in Fig. 5 might be taken as a premise upon the slight stimulation of the photosynthesis (about 40% increasing) in the case of low magnetic exposure times (0.5–1–2 hours). The statistical analysis accomplished for the chlorophyll ratio (by applying the *t*-test to compare control and test sample data) revealed statistic significance ($p < 0.05$) for all exposed samples.

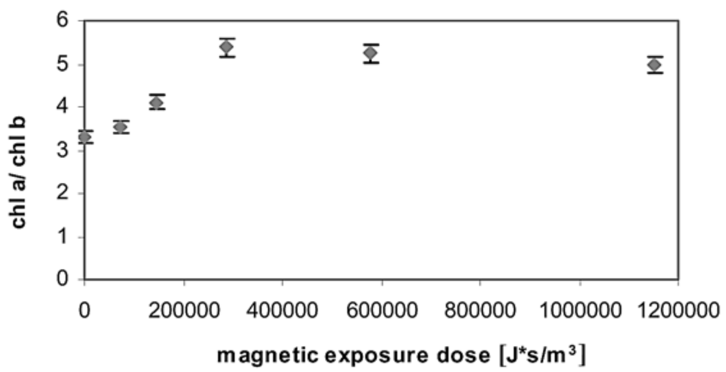


Fig. 5 – The effects of extremely low frequency magnetic field exposure on chlorophylls ratio.

These results provide also a suggestive picture for the slight inhibitory effect of extremely low frequency magnetic field in the case of 4 and 8 hours exposure times. This is concordant with the effect observed in the case of chlorophyll a (Fig. 3), which is the most important assimilatory pigment involved directly in the conversion of solar energy into chemical energy at the molecular level in the frame of photosynthesis complex phenomena. The average content of nucleic acids in young *Robinia pseudoacacia* seedlings after the extremely low frequency magnetic field exposure is presented in Fig. 6. One can see that for increasing magnetic exposure time (dose) the average nucleic acid level is diminished in comparison to the control sample. Applying the *t*-test to compare control and test sample, data for the average nucleic acid level have revealed statistic significance ($p < 0.03$) for all exposed samples.

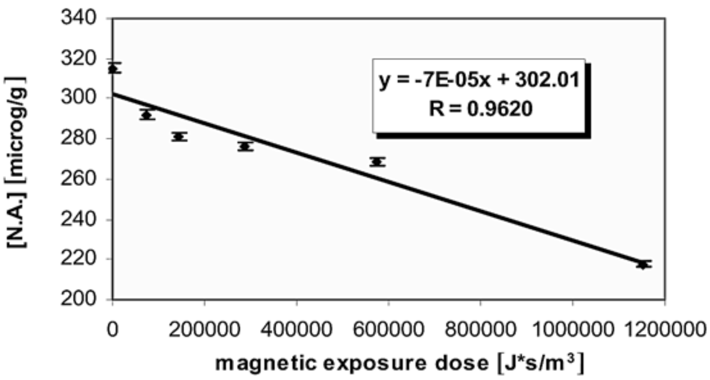


Fig. 6 – Average nucleic acid level in leaves of young *Robinia pseudoacacia* seedlings.

4. CONCLUSIONS

In summary, in this preliminary study regarding extremely low frequency magnetic field influences upon arbor seedlings, during their early ontogenetic stages, slight stimulation of the photosynthesis process was revealed by means of chlorophylls ratio (about 40% increasing) in the case of low times (0.5–1–2 hours) of magnetic exposure. Also, a negative dependence of the magnetic exposure on the nucleic acid biosynthesis in young *Robinia pseudoacacia* seedlings on the time was obtained.

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