THE RESPONSE OF PLANT TISSUES TO MAGNETIC FLUID AND ELECTROMAGNETIC EXPOSURE

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Abstract. This experimental study was dedicated to the possible oxidative stress induced by magnetic fluid accumulation in the living tissues. The aqueous magnetic fluid was prepared by dispersing iron oxide particles coated with perchloric acid in water, characterized by physical diameter average value of 10.55 nm as revealed by electron microscopy and 18.3 kA/m value for saturation magnetization. Electromagnetic exposure was accomplished during the seeds germination, within transverse electromagnetic cell at 20 mW power level and 300 kHz frequency for 30 minutes while diluted magnetic fluid was supplied both during germination and plant development. The biochemical investigations, i.e. the assay of photo-assimilatory pigments and nucleic acids contents, after 12 days of plants growth were performed. Chlorophylls ratio was diminished up to 30% while plant lengths were shorter with about 40% in the samples exposed to electromagnetic field; however, nucleic acid biosynthesis seems to be remarkably stimulated following the electromagnetic exposure.

Key words: magnetic nanoparticles, EMF exposure, *Zea mays*, hyperthermia, chlorophylls, nucleic acids.

INTRODUCTION

There are various scientific reports presenting either plant response to electromagnetic exposure [2, 6, 9] or to magnetic nanoparticle supply [3, 11, 13] but we found only few titles regarding the influence of both these factors on technical plant growth [1]. Magnetic fluids generally consist of mono-domain magnetic nanoparticles dispersed in water or a hydrocarbon fluid [12]. For bio-medical applications, the biocompatibility of both the fluid and nanoparticles must be considered, thus the fluid and magnetic materials should not be toxic. The established biocompatibility of magnetite (Fe_3O_4) makes it a common choice. The magnetic particles should remain evenly dispersed throughout the carrier fluid, and must therefore have small size to avoid precipitation due to gravitational forces.

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Ferro- and ferromagnetic particles display magnetism even in the absence of an applied magnetic field [7]. Magnetic particles with diameters around 10 nm typically demonstrate superparamagnetic properties. The magnetic moments of superparamagnetic nanoparticles are randomly reoriented by the thermal energy of their environment and do not display magnetism in the absence of a magnetic field. An applied alternating magnetic field can provide the energy necessary to reorient the magnetic moments of the magnetic particles. This magnetic energy, when dissipated, is converted to thermal energy [10]. In addition to causing changes in the magnetic moments, this energy can force the magnetic nanoparticles to physically rotate.

Previous researches about magnetic fluid influence on bio-organisms showed that plant metabolism could be influenced by magnetic nanoparticles addition in culture medium, which is able to induce phenotypic and genotypic effects resulting in possible stimulation of the productivity [8, 14]. It is believed that the iron oxides provided by the magnetite from magnetic fluid ferrophase could interfere with the complex redox reactions involved in the photosynthesis phenomenon. The small size of nanoparticles, between 8 and 10 nm, suggests the ability of bio-membrane penetration for small diameter values or the fact that these could remain embedded in biomembranes or in the cell cellulose wall, so that their superparamagnetic properties could influence locally the transmembrane ion flows [4].

The biological interest in the magnetic fluid effect in living organisms represents an important application field of magnetic nanoparticles, mainly for biotechnological use. In this paper the authors present some quantitative observations regarding the influence of magnetic nanoparticles and electromagnetic field on the growth of *Zea mays* plants in early ontogenetic stages.

MATERIALS AND METHODS

The present experimental study was focused on the assimilatory pigments and nucleic acid levels in young plants intended for agricultural use (*Zea mays*), provided by germinated seeds in magnetic fluid and electromagnetic field presence, grown in the presence of water based magnetic fluid in culture medium.

The water based magnetic fluid was constituted by coating the small magnetic nanoparticles with perchloric acid and water as carrier liquid. Saturation magnetization of our magnetic fluid was of 18 kA/m; the magnetic nanoparticles volume fraction was of 4.5% while the average magnetic particle size was equal to 10.55 nm. The ferrophase content was of $2.03 \cdot 10^{17}$ particles within 1 mL of the initial magnetic fluid. Seeds with a uniform genofond were let to germinate in controlled environmental conditions (darkness and suitable temperature) into a culture room, on porous paper support impregnated with the same amount of magnetic fluid suspension (after vigorous shaking) in four different volume fractions in Petri dishes (each sample was compounded of 50 seeds). Volume fractions of water based magnetic fluid were of: 50; 100; 200 and 300 microliter

magnetic fluid per liter of deionized water (μL/L). After the sprouting of seeds, for each volume fraction of magnetic fluid solution used in this experimental study we arranged an experimental sample that was exposed to electromagnetic field of low power density, for 30 minutes within a TEM cell. Continuous wave on 300 kHz frequency with an input power of 20 mW was transmitted inside the TEM cell. Thus, we arranged two experimental variants: LM samples – germinated seeds in the presence of magnetic fluid, and LM-EMF samples – germinated seeds in the presence of magnetic fluid and exposed for 30 minutes to electromagnetic field. It was also a control sample. The Petri dishes containing seeds were exposed one by one in the same position inside a TEM cell (model IFI CC-104SEXX), which was supplied from a signal generator (model Hameg HM 3184-3) (Fig. 1). Incident field distribution in the irradiation area was characterized as uniform field to be applied in the volume of the samples.

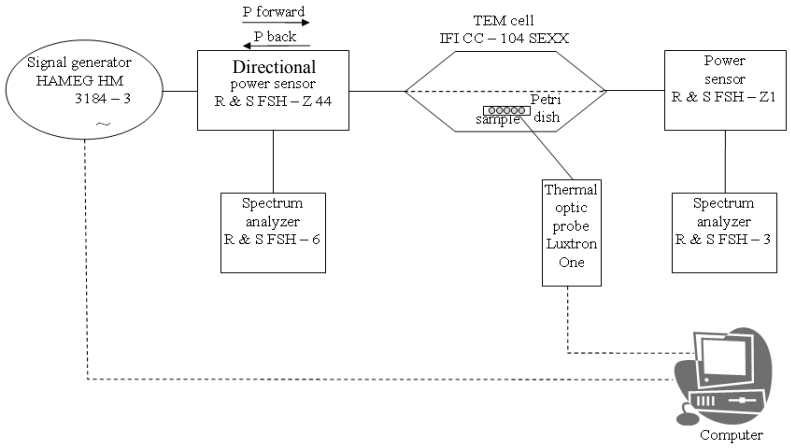

Fig. 1. Exposure set-up scheme.

During plant sample growth daily supply was carried out with 10 mL magnetic fluid aqueous suspension per dish of sample for 12 days, in controlled conditions of temperature (22.0 ± 0.5 ºC), illumination (dark/light cycle: 14 h / 10 h) and 90% humidity into a culture room. The Lichtenthaler & Wellburn's method [5] was used to assay the assimilatory pigments and a modified Spirin's method [15] for nucleic acids assay after 12 days of plant growth.

Biological material consisted of green tissue obtained by mixing up the green tissue from the all young plantlets grown from each experimental sample. The spectral device was a CINTRA 5 spectrophotometer UV-VIS provided with quartz cells. Three repetitions of experimental investigations about assimilatory pigments and nucleic acids extraction and spectrophotometric assays were carried out for all experimental variant samples. Average values, standard deviations and t-test have been considered for statistical analysis. Plant individual length was measured with

0.1 cm precision and statistical analysis was accomplished by means of average plant lengths, standard deviation and confidence interval, calculated for each batch of plantlets using the Student t-test.

RESULTS

The lengths of the 12 days old plantlets were carefully measured with 0.1 cm precision. The average lengths and the standard deviations were calculated for each batch of test seeds. The confidence interval was calculated for every batch of plantlets using the Student test, for the confidence level p = 90%, too.

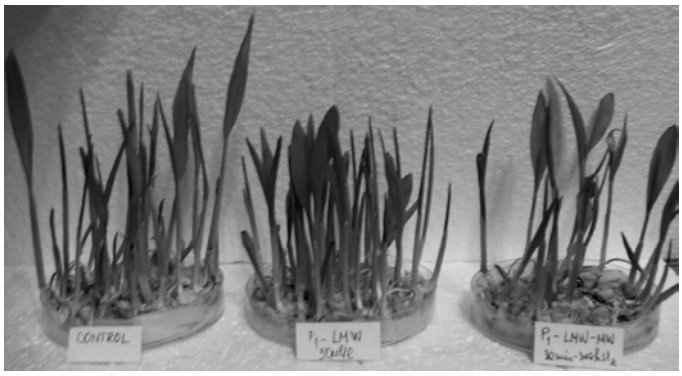

Fig. 2. Some of the 8 days old *Zea mays* plant samples. Control sample – left, P_1 sample – 50 µL/L magnetic fluid supplied (LM) – center, P_1' sample – 50 µL/L magnetic fluid supplied and electromagnetic field exposed (LM-EMF) – right.

Fig. 3 presents the average plants length for all experimental samples. For both series of samples, either magnetic fluid supplied or magnetic fluid supplied and electromagnetically exposed, we found that relatively high volume fractions of the magnetic fluid solution have an inhibitory effect on the plants growth. The inhibitory effect seems to be deeper for the second plant series – up to about 40% – where the electromagnetic exposure could also influence plant growth.

We hypothesize that at the electromagnetic field exposure moment could produce a process like that of hyperthermia. This might cause a local heating of the vegetal tissue due to small magnetic nanoparticles internalized in vegetal tissue. This local heating of the vegetal tissue could affect the plant metabolism.

The contents of photosynthesis pigments (**a** and **b** chlorophylls and total carotenoids) in the green tissue of young *Zea mays* plantlets (aged 12 days) for experimental samples are presented in Figures 4, 5 and 6.

The chlorophyll **a** level, the main photosynthesis pigment, was found to be slightly increased for enhanced volume fraction of magnetic fluid solution, in the LM samples cases, thus a slow stimulatory effect (of about 30%) was noticed (Fig. 4).

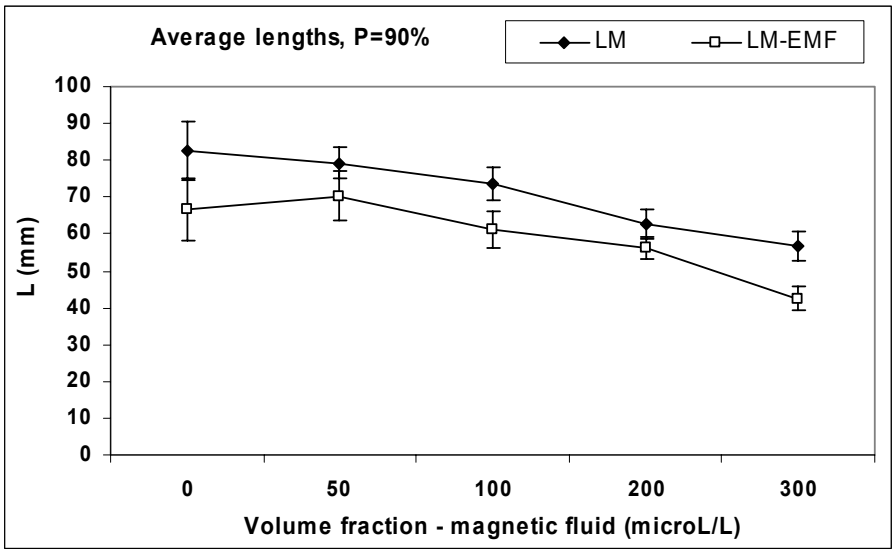

Fig. 3. The average length versus volume fraction of magnetic fluid solution added in culture medium, LM – magnetic fluid supplied samples, LM-EMF – magnetic fluid supplied and electromagnetic field exposed samples.

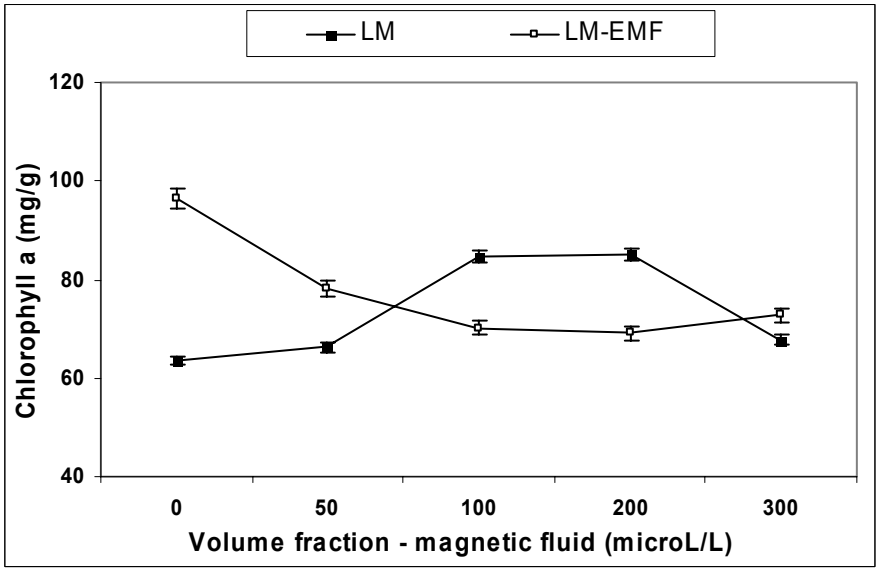

Fig. 4. The chlorophyll ***a*** contents versus volume fraction of magnetic fluid solution added in culture medium, LM – magnetic fluid supplied samples, LM-EMF – magnetic fluid supplied and electromagnetic field exposed samples.

Similar response was obtained for the other all photo-assimilatory pigments analyzed (Figs. 5 and 6).

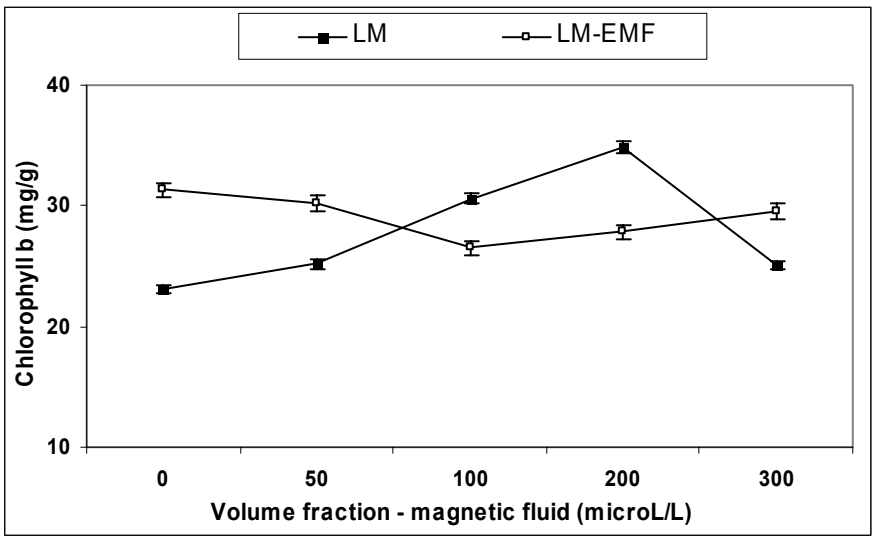

Fig. 5. The chlorophyll **b** contents versus volume fraction of magnetic fluid solution added in culture medium, LM – magnetic fluid supplied samples, LM-EMF – magnetic fluid supplied and electromagnetic field exposed samples.

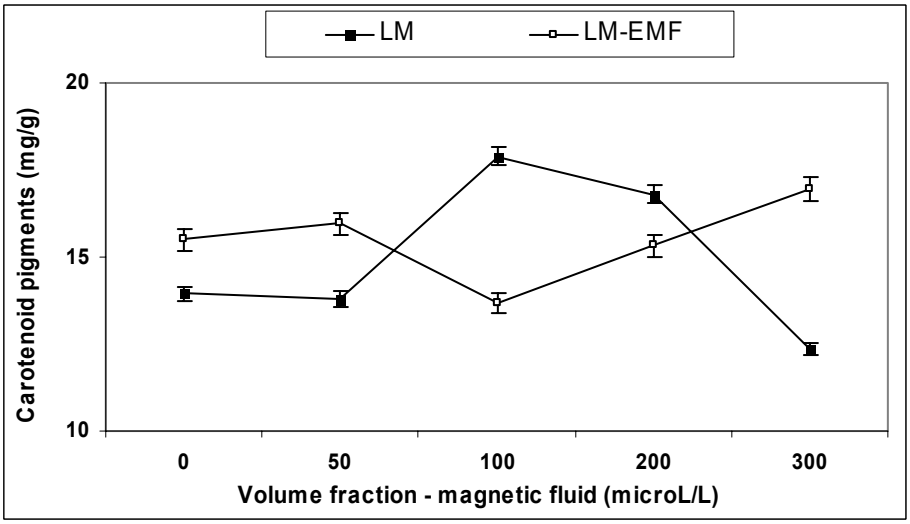

Fig. 6. The carotenoid pigments contents versus volume fraction of magnetic fluid solution added in culture medium, LM – magnetic fluid supplied samples, LM-EMF – magnetic fluid supplied and electromagnetic field exposed samples.

For the LM-EMF experimental samples, the assimilatory pigments content decreased – with about 20% in the case of chlorophyll **a** – when the volume fraction of magnetic fluid solution increased, an inhibitory effect being evidenced. The effect might be due to a local heating of the vegetal tissue caused by the energy absorbed by the magnetic nanoparticles internalized in vegetal tissue. This local heating of the vegetal tissue could affect the redox reactions implicated in the photosynthesis process.

The chlorophylls ratio (chlorophyll **a** / chlorophyll **b**) is considered the best indicator upon the photosynthesis process efficiency [16] which provides indirect information on the activity of the enzymatic aggregates of the Light Harvesting Complex II (LHC II) from the photosynthetic system II located in the chloroplasts membranes.

In Fig. 7 the inhibitory influence of increased volume fractions of magnetic fluid solution – up to about 30% in the case of electromagnetic exposure, upon the photosynthesis process is visible, as suggested by chlorophyll **a** and **b** ratio.

The total content of nucleic acids (DNA+RNA) in young *Zea mays* plants after 12 days of growth under different volume fraction of magnetic fluid solutions added in plants culture medium for all experimental samples is presented in Fig. 8.

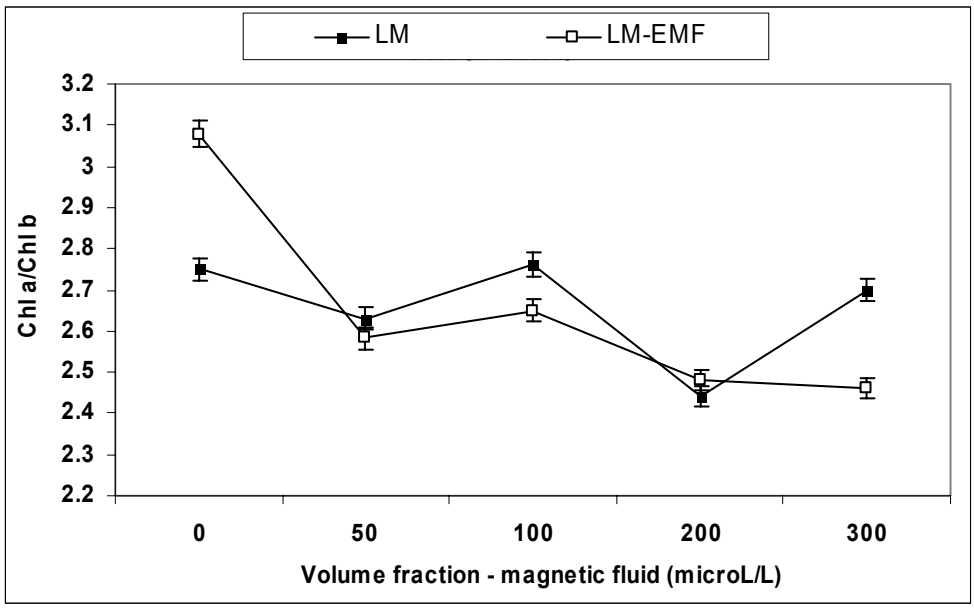

Fig. 7. The chlorophylls ratio versus volume fraction of magnetic fluid solution added in culture medium, LM – magnetic fluid supplied samples, LM-EMF – magnetic fluid supplied and electromagnetic field exposed samples.

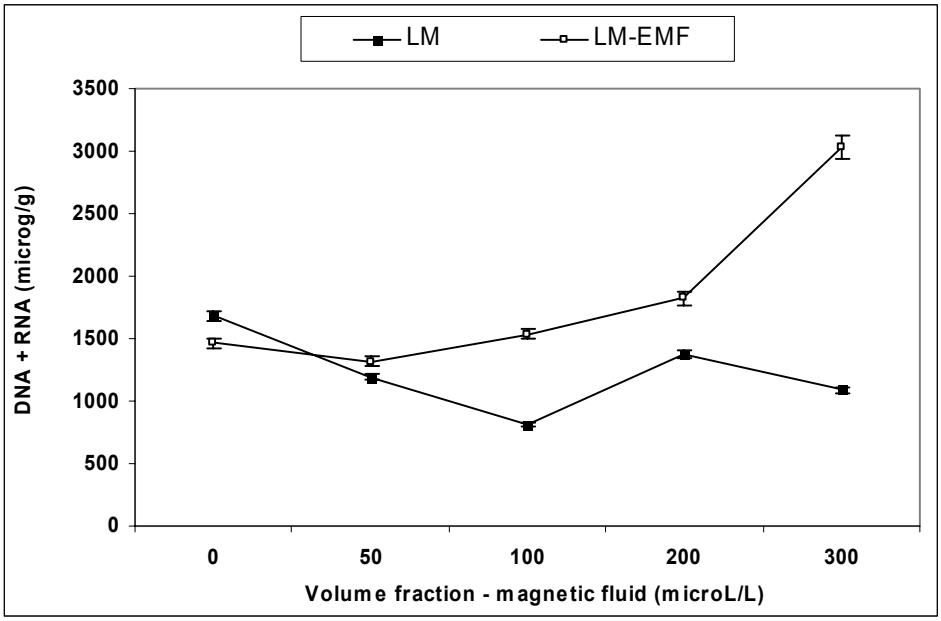

Fig. 8. The nucleic acids level versus volume fraction of magnetic fluid solution, LM – magnetic fluid supplied samples, LM-EMF – magnetic fluid supplied and electromagnetic field exposed samples.

For increasing volume fraction of magnetic fluid solution we found an average nucleic acid level decreased in comparison to the control sample, revealing an inhibitory effect on biosynthesis.

Since a presumption of magnetic fluid supply interference with the nucleic acid biosynthesis is needed, one could imagine that the ferrophase could penetrate the nuclear membrane but the existence of extra-nuclear DNA and RNA need to be also taken into account.

In the LM-EMF experimental samples case we obtain a stimulatory effect on nucleic acids level (Fig. 8). We hypothesize that this response is due to regeneration reactions of the plant metabolism processes against the putative local heating of the vegetal tissue produced by the electromagnetic field energy absorbed by the magnetic nanoparticles internalized in vegetal tissue.

CONCLUSIONS

The present experimental study was focused on the assimilatory pigments and nucleic acid levels in young plants intended for agricultural use (*Zea mays*), provided by germinated seeds in magnetic fluid and electromagnetic field presence, grown in the presence of water based magnetic fluid in culture medium.

The inhibitory effects obtained for the plants grown from the seeds germinated in the magnetic fluid presence and then exposed to electromagnetic field during the germination process (LM-EMF samples) are higher than for the plants grown from the seeds germinated in the magnetic fluid presence but in the lack of electromagnetic exposure (LM samples). For the LM-EMF samples, the assimilatory pigments contents were found decreased for increased volume fraction of magnetic fluid solution – with 20% in the case of chlorophyll **a**, an inhibitory effect being evidenced. We can assume that the electromagnetic field exposure moment could produce a process like that of hyperthermia a local heating occurring due to the electromagnetic field energy absorbed by the magnetic nanoparticles internalized in vegetal tissue. This local heating of the vegetal tissue could affect the redox reactions implicated in the photosynthesis process.

A stimulatory effect on nucleic acids biosynthesis was obtained in the LM-EMF experimental samples – a twice higher level than in the control samples. We believe that this response is due to regeneration reactions of the plant metabolism processes against the putative local heating of the vegetal tissue produced by the electromagnetic field energy absorbed by the magnetic nanoparticles internalized in vegetal tissue.

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