

# INHIBITORY EFFECTS OF LOW THERMAL RADIOFREQUENCY RADIATION ON PHYSIOLOGICAL PARAMETERS OF *ZEA MAYS* SEEDLINGS GROWTH

M. RACUCIU<sup>1</sup>, C. IFTODE<sup>2</sup>, S. MICLAUS<sup>3</sup>

<sup>1</sup>Department of Environmental Sciences and Physics, Faculty of Sciences, Research Center on Applied Ecology, "Lucian Blaga" University of Sibiu, Romania, E-mail: mracuciu@yahoo.com

<sup>2</sup>Faculty of Electronics and Telecommunication Engineering, Politechnica University, Timisoara, Romania, E-mail: cora.iftode@gmail.com

<sup>3</sup>Department of Technical Sciences, "Nicolae Bălcescu" Land Forces Academy, Sibiu, Romania  
E-mail: simo.miclaus@gmail.com

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12-days old plantlets developed from *Zea mays* seeds exposed at 1GHz inside a transverse electromagnetic cell for different exposure durations, at a specific absorption rate of energy deposition of 0.47W/kg, showed decreased levels of photo-assimilatory pigments and nucleic acid contents, than controls. Longer exposure duration inhibited the plantlets' growth.

*Key words:* radiofrequency radiation, low thermal effect, *Zea mays*, photo-assimilatory pigments, nucleic acids.

## 1. INTRODUCTION

The scientific interest on the effects of high frequency electromagnetic fields on biological materials, especially plants, dates since the 19th century [1]. Not a few of the earlier experiments on plant material have been focused on the effect of radio frequency electromagnetic fields on seeds. In many cases, the short exposure resulted in increased germination rate and stimulation of seedlings growth [2]. Ponomarev *et al.* [3] investigated the influence of low intensity microwave radiation on the germination of cereals (winter and spring wheat, spring barley, oats), observing an increasing of germination rate for all the treated seeds. Khalafallah *et al.* showed in their experiment that the germination grains and growth rate of exposed maize seedlings, significantly increased compared to the control.

Also, photosynthetic pigments levels, total soluble sugar and total carbohydrates were positively affected by 945 MHz electromagnetic field exposure [4]. Jonas reported that the action of microwave radiations on *Zea mays* seedlings damaged the photosynthetic system and led to significant increase of the carotene and anthocyanin production [5]. Exposing *Lemna minor L.* plants to 900 MHz

electromagnetic field, Tkalec *et al.* have observed a decreasing of the plants growth for 2 hours exposure, even if the germination rate and the root lengths not changed significantly [6]. M. Ursache *et al.* [7] exposed *Zea mays* seedlings at 418 MHz electromagnetic field for relatively short exposure times (1 to 4 hours), and observed increasing of the photosynthetic pigments levels.

L. Oprică [8] has studied microwave treatment with power density under  $1 \text{ mW/cm}^3$  on rape seeds and concluded that the exposure determined variations of catalase and peroxidase levels that depend on the age of the plants and time of exposure. Sandu *et al.* [9] studied the 400 MHz electromagnetic field influence on the black locust (*Robinia pseudoacacia*) seedlings. Chlorophyll a and chlorophyll b levels were found to decrease and chlorophylls ratio was decreased logarithmically to the increase of daily exposure time on the electromagnetic field. Roux *et al.* [10] showed that a non-thermal 900 MHz electromagnetic field on tomato plants was able to evoke rapid accumulation of some transcripts (like  $\text{Ca}^{2+}$  receptors) that are known to play an important role in the plant responses to stress.

The present study aimed to quantify the effects of low-power 1GHz electromagnetic field action on *Zea mays* seeds, by assessing of the photosynthetic pigments and nucleic acid total level in seedlings developed from exposed seeds. The seeds exposure took place in a well-controlled environment and was followed by a proper microwave dosimetric analysis.

## 2. MATERIALS AND METHODS

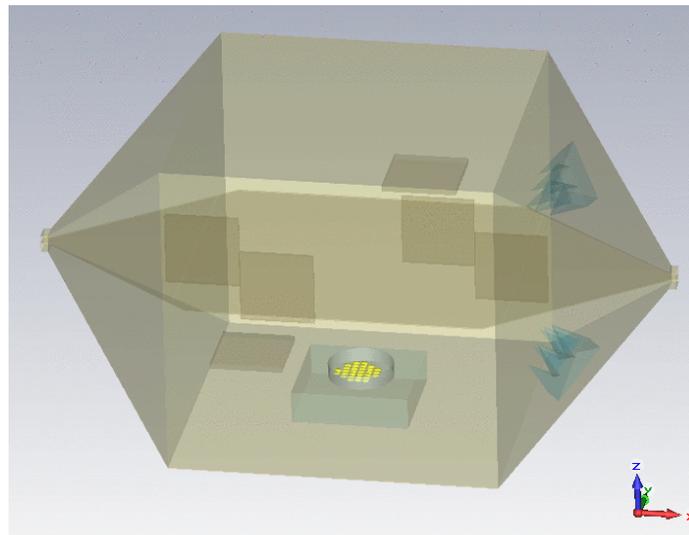
### 2.1. BIOLOGICAL MATERIAL

Considering its economic importance for agriculture and food industry the popcorn seeds (*Zea mays*) were chosen as biological material. In order to diminish the putative genophond variations in this experiment were used seeds from a single plant with vigorous biological features from an experimental micro-population. Irradiated seeds germination occurred on porous paper support in closed Petri dishes, environmental conditions being kept under peer control (temperature being  $24 \pm 0.50 \text{ degC}$ , 98% humidity, in darkness). After germination the young plantlets development was realized in the same controlled laboratory conditions ( $23 \pm 0.50 \text{ degC}$ , illumination -11h: 13h light/dark cycle and 90% humidity) and the culture medium of young plantlets was daily watered with the same amount of de-ionized water.

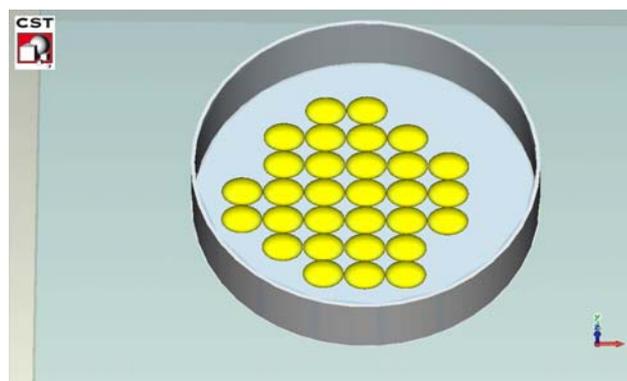
### 2.2. EXPOSURE SYSTEM AND MODELING-SIMULATION

Biological samples composed of 30 *Zea mays* seeds each, having a uniform genophond, were exposed one by one (in a Petri dish) to 1GHz frequency microwave, inside a transverse electromagnetic (TEM) cell, for different exposure

times between 1 hour and 8 hours (Fig. 1). The TEM cell was model IFI CC-104SEX (Instruments for Industry, USA) and at the input port the incident power  $P_{in}=11.5W$  was delivered from a radiofrequency signal generator model Hameg HM 3184-3 through a power amplifier Ophir 5150. At the output port the cell was terminated by 50 ohm matched impedance. In this way, a relatively uniform electric (E) field strength distribution was obtained in a large volume between the floor and the septum of the TEM cell [11], at the place where the sample was exposed.



(a)



(b)

Fig. 1 – The modeled TEM cell and the disposal of the corn seeds sample inside it, for uniform exposure to 1GHz/11.5W: a) the position of the Petri dish inside the cell during the exposure; b) the disposal of the seeds inside the dish.

A modeling-simulation step followed, in order to obtain the specific absorption rate of energy deposition (SAR) in the sample. The validation of the approach [11] showed that in general a good agreement between measured and simulated SAR is obtained. In present case, the simulation was made in CST Microwave Studio. The simulated TEM cell and the sample disposal inside it is observed in Fig. 1a, while in Fig. 1b the distribution of the seeds in the Petri dish during exposure is indicated. Getting primary data from the real sample, each corn seed was then modeled as a little ellipsoid, with the axes of  $1 \times 0.8 \times 0.41$  cm, and with an average mass of 0.317 g. The mass density of a corn seed was of  $1.8628 \text{ g/cm}^3$ . The dielectric properties of the seeds were extracted from [12], by considering an average of 23% relative humidity of the sample. So, the dielectric data for *Zea mays* were: relative permittivity real part - which is related to the stored energy, was  $\epsilon'_r = 7.15$ , relative permittivity – imaginary part, which is related to the dissipation (or loss) of energy was  $\epsilon''_r = 1.52$ , and the electric conductivity was  $\sigma = 84.5 \times 10^{-3} \text{ S/m}$ .

### 2.3. ANALYSIS METHODS

The assimilatory pigments (chlorophyll a, chlorophyll b and total carotenoid pigments) and average nucleic acids (DNA and RNA) levels in the green tissues of all experimental samples as well as in the control plants, after the 12 days of plant development, were assayed by spectrophotometric methods using a JASCO V530 spectrophotometer UV-VIS device provided with quartz cells of 1cm width. Using the Lichtenthaler and Welburn's method [13], the assay of the assimilatory pigments extracts (in 80% acetone) was performed, while the assay of nucleic acid level (in perchloric acid 6% extracts) was carried out accordingly to modified Spirin's method [14, 15]. Spectrophotometric measurements were performed at the wavelengths of: 663nm, 646nm and 470nm (for 80% acetone extracts) for the assay of chlorophylls (Chla, Chlb) and carotenoids pigments (Car) from green tissues and, at 260nm and 280nm (for 6% perchloric acid extracts) in the case of nucleic acids. For obtaining of photosynthetic pigments levels, the formulas from Lichtenthaler and Welburn [13] were applied while the calibration curves (based on the spectral readings to the mentioned wavelengths) were used in the case of nucleic acids. The biological material, used in the analysis conducted in this study, was consisted of green tissue obtained by mixing up the green tissue from the all young plantlets grown from each experimental group (sample). It was used the same amount of fresh green tissue mass for each experimental sample. Plant individual length was measured with 0.1cm precision and the average lengths and the standard deviations were calculated for each batch of test seeds. Using the Student test, the confidence interval was calculated for every batch of plantlets for the confidence levels  $P = 90\%$ ,  $95\%$  and  $99\%$ . Since chlorophylls ratio revealed the

response of the LHC II system (Light Harvesting Complex II) to the external agents, these experimental data offered the main insight into the photosynthesis complex processes [16].

#### 2.4. STATISTIC ANALYSIS

Statistic analysis of the experimental data, resulted from the three repetitions of the whole experiment, was accomplished by means of ANOVA test - applied using MsExcell soft package - to evaluate reliability of modifications induced by electromagnetic field exposure in comparison to the control ones as well as among the samples corresponding to different exposure time, considering the significance criterion of 0.05 (p value).

### 3. RESULTS

The SAR distribution, obtained in the simulation (Fig. 2), was not very uniform varying between 0.27–0.65 W/kg, with an average value  $SAR_{avg} = 0.47$  W/kg. With this SAR value, and by considering a specific heat of the corn  $c = 3350$  J/kg.degC, an expected temperature increase due to microwave exposures between 0.50 degC and 4.04 degC is expected, when no heat exchange would take place between the seeds sample and the environment. This would conduct to a low-thermal effect.

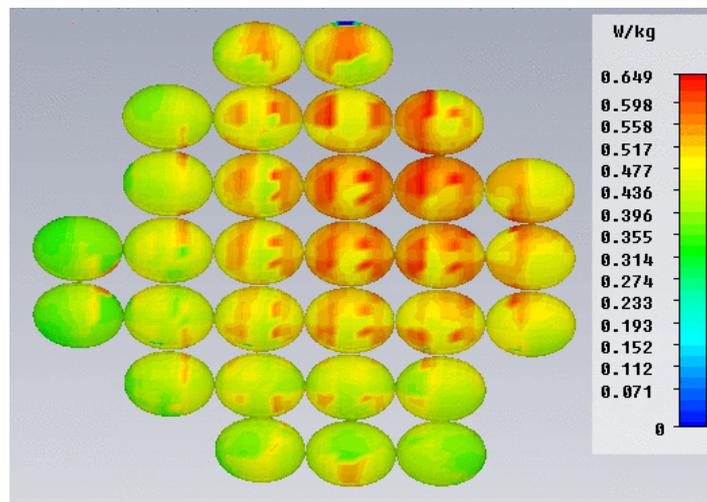


Fig. 2 – SAR distribution along the 30 seeds probe exposed in the TEM cell at 1GHz.

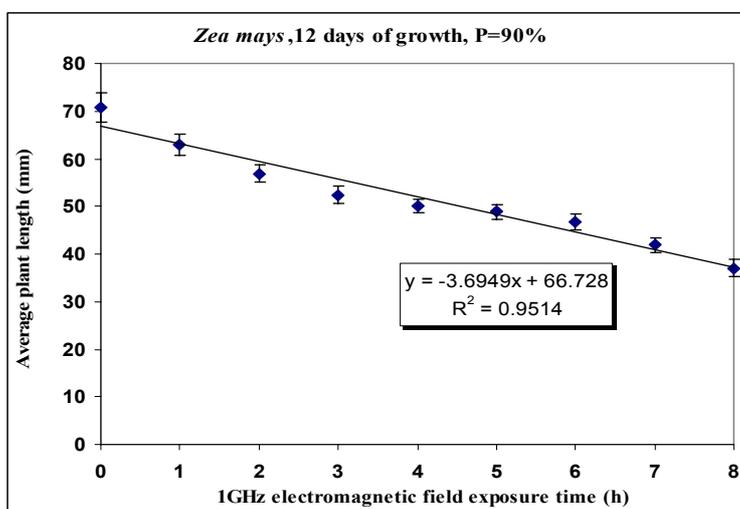


Fig. 3 – The average length of 12 days old plantlets in function of exposure time.

The average lengths of plantlets and afferent standard deviations were calculated for each batch of test seeds and represent in Figure 3 in function of electromagnetic field exposure time. It was found an inhibitory effect on plant growth under the 1GHz electromagnetic field action with enhanced exposure times, observing a linear correlation with the exposure time. The confidence interval was calculated for every batch of plantlets using the Student test, for the confidence level  $P = 90\%$ . All length plantlet results are statistically significant in comparison to control.

The photosynthesis pigments levels (a and b chlorophylls and total carotenoids) in the green tissue of young *Zea mays* plantlets (aged of 12 days) for experimental samples are presented in Fig. 4. The chlorophyll a content, the main photosynthesis pigment, was found decreased for all electromagnetic field exposure times used in this experiment comparatively to the control sample (plants growth was performed only in deionised water presence) (statistically significant in relation to the threshold of 0.05).

The total assimilatory pigments contents had the same variation to the increase of exposure time of electromagnetic field action on 12 days young plantlets that was observed for chlorophyll a level. The chlorophylls ratio (chlorophyll a/chlorophyll b) is considered the best indicator upon the photosynthesis process efficiency [16], which provides indirect information on the enzymatic aggregates of the LHC II (Light Harvesting Complex II) from the photosynthetic system II located in the chloroplasts membranes. A slight variation of chlorophylls ratio was observed for enhanced exposure time only for the greater exposure time (8 hours) was observed decreased value, with 22% than unexposed sample value (statistical significance was ensured relatively to the threshold of  $p < 0.05$ ) (Fig. 5). From

results, it was observed for relatively small exposure times a tendency to accumulation of more chlorophylls than carotenoids while for enhanced exposure times (between 4 and 8 hours) this tendency has been changed, observing an accumulation of more carotenoids than chlorophylls at plant level. Chlorophylls to carotenoids ratio decreased with 1GHz electromagnetic field exposure time (Fig. 6).

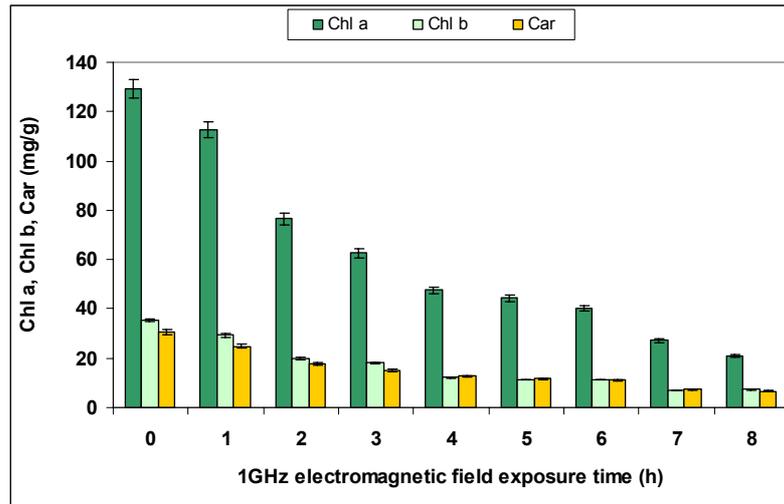


Fig. 4 – Photo-assimilatory pigments level in *Zea mays* plantlets versus 1GHz electromagnetic field exposure time. (Chl a – the content of chlorophyll a, Chl b – the content of chlorophyll b, Car – the content of total carotenoid pigments).

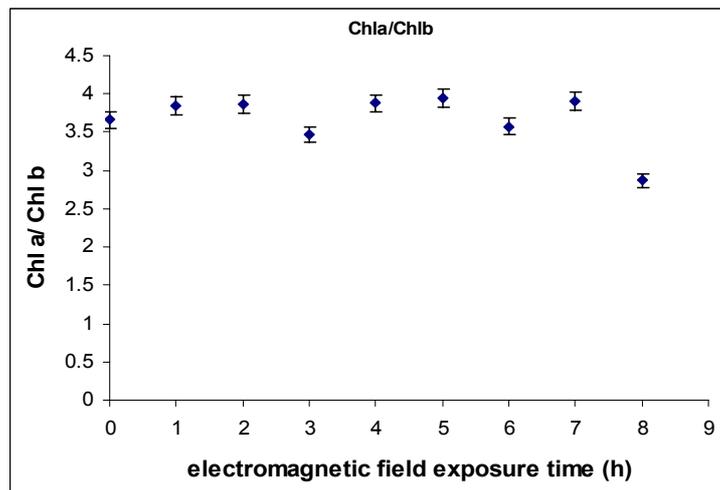


Fig. 5 – The effects of electromagnetic field exposure on chlorophylls ratio (Chl a/Chl b).

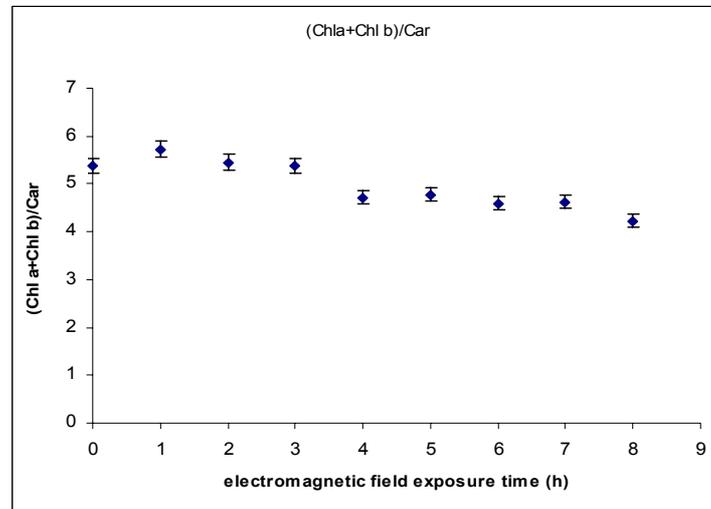


Fig. 6 – (Chl a+Chl b)/Car level for the plantlets provided by electromagnetic field exposed seeds.

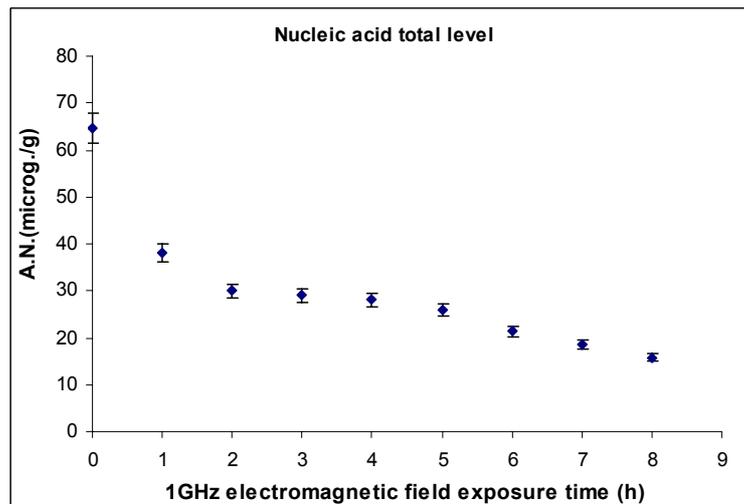


Fig. 7 – The level of DNA and RNA for the plantlets provided by electromagnetic field exposed seeds.

The nucleic acids average content in young *Zea mays* plantlets after 12 days of grown developed from 1GHz electromagnetic field exposed seeds is presented in Fig. 7. It was observed that for increasing electromagnetic field exposure time the nucleic acid biosynthesis was inhibited (about 75 %) in comparison to the control sample (plants developed from unexposed seeds). Applying the t-test to compare control and test sample, data for the average nucleic acid level statistic significance ( $p < 0.05$ ) was found for all samples. Nucleic acid total level

decreased exponentially with exposure time  $t$ , experimental fitting curves following  $20.72 + 42.37e^{-t/1.44}$  equation with  $R^2 = 0.94227$ .

#### 4. CONCLUSION

In summary, in this experimental study regarding the 1GHz frequency radiation exposure influences upon plant seedlings developed from exposed seeds, during their early ontogenetic stages, an inhibitory influence on the plants growth was observed, the average plants length values being diminished for all exposure times.

The results of the biochemical analysis carried out on *Zea mays* plants, developed from 1GHz radiation exposed seeds, during their early ontogenetic stages, have revealed that plant development could be influenced during such treatment. For photo-assimilatory pigments level of *Zea mays* plantlets, an inhibitory effect was observed for all exposure times, also. A slight variation of chlorophylls ratio in *Zea mays* plantlets developed from exposed seeds was observed for all exposure time with one exception (for the greater exposure time-8 hours), when it was observed a decreased value. Also, the total level of DNA and RNA of plantlets developed from exposed seeds was significantly decreased than control sample level. Therefore, the exposure times between one and eight hours of 1GHz frequency radiation on *Zea mays* seeds have disruptive effects at plantlets level, developed from exposed seeds, regarding the young plant growth, photo-assimilatory pigments and nucleic acids contents.

In our experiments it was observed also, that the 1GHz frequency radiation exposure is able to initiate a mutagenic effect and an inhibition of the cellular proliferation and differentiation in the radiofrequency radiation exposed seeds in comparison to the control sample. These modifications could be observed further on in the adult plant development since the inhibited mitotic process in the root tissues will lead to a slow development of plants or to occurrence of genetically modified plants.

In the near future, will be designed such new experiments to support new hypotheses on the mechanism of radiofrequency electromagnetic field influence on vegetal organisms.

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