CONSIDERATION ON THE PEROXIDASE ACTIVITY DURING HIPPOPHAE RHAMNOIDES SEEDS GERMINATION EXPOSED TO RADIOFREQUENCY ELECTROMAGNETIC FIELD INFLUENCE

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Abstract. The data accumulated by now shows that the topic of biological effects of electromagnetic radiation is far from being exhausted. Is undoubtedly that a non-ionizing radiation field maintained on a biological entity has some effects on it. To try shaping issues regarding this, this work aims to study the impact of radiation generated by an emission-reception radio station that emits on 462.6875 MHz frequency. For this purpose, were used *Hippophae rhamnoides* L. seeds which germinated in the laboratory, under controlled conditions, concentrically arranged around the radiation source, in which case electromagnetic radiation has a different impact. Seed germination lasted 35 days, while the device has continuously worked, and the seeds were constantly irradiated. The intensity of the magnetic component of the field was precisely measured in all places where the seeds were placed for germination. It was calculated the percentage of germination and it was determined the peroxidase activity involved in eliminating the oxidative stress effects. Accordingly to the distance from the source, significant variations of the parameters mentioned above in conjunction with the radiation intensity were found.

INTRODUCTION

Accelerated and widespread use of different electric and electronic devices increased the exposure to radio and microwave frequency electromagnetic fields (EMFs). These EMFs are classified as non-ionizing radiation but they can cause damage depending on the power level, frequency, and the properties of exposed tissue. There is some evidence that microwaves (300 MHz–300 GHz) produces changes in the cell membrane’s permeability and cell growth rate as well as interference with ions and organic molecules, like proteins (Kwee et al., 1998, 2001; de Pomerai et al., 2003; Repacholi, 2001; Pologea-Moraru et al., 2002; Banik et al., 2003). Plants are essential components of a healthy ecosystem and have important role in the living world as main primary producers of food and oxygen; therefore it would be beneficial to investigate their interaction with today’s increased exposure to radio and microwave frequency fields. Additionally, higher plants are useful test organisms for environmental studies because they are eukaryotic multicellular organisms. Many of them are sensitive to different kinds of stresses and are easy to grow in controlled laboratory conditions without too much expense (Wang, 1991). During the years it became more and more interesting to test the effects of EMFs on higher plants (Tkalec et al. 2005, 2007). Considering the increasing interest for the subject, this work focus on the influence of 462.6875 Mhz EMF on the oxidative stress during the *Hippophae rhamnoides* seeds germination. This species was chosen because of the following aspects. Firstly, the period of germination is relatively long, the experiment is held over a period of 35 days, this issue was important because the seeds were irradiated for a long time, unlike other species that germinate very fast (3-5 days). Secondly, sea buckthorn (*Hippophae rhamnoides* L.) is a species which has some interesting biochemical characteristics: vitamins B, C, E, K, carotenoids (the most dominant carotenoid in sea buckthorn, it’s admitted to be associated with reduced risk of breast, stomach, esophageal, and pancreatic cancers), flavonoids (it have been found in controlling arteriosclerosis, reducing cholesterol level, turning hyperthyroidism into euthyroidism and eliminating inflammation), tannins, metallothionein (acts as detoxifying agency for heavy metals and as free radical scavenger for most toxic radical) and 5-hydroxytryptamine (5-HT), a chemical neurotransmitter substances (Lian, 2000; Thomas, 2003).

MATERIALS AND METHODS

To seek evidence of the influence of electromagnetic field (EMF) of radio frequency on oxidative stress, during the germination of seeds, was used a source consisting of two Motorola T5725 emission-reception radio stations that have been programmed to automatically call one another throughout experiment. The communication system frequency is set on channel 6 at 462.6875 MHz with 500mW transmit power. Thus, around the two emission-reception radio stations were delimitated four concentrically levels (different distances from the source), with four groups with five Petri Dishes (A1-A5; B1-B5; C1-C5; D1-D5), in each plate with about 20 seeds. The control lots, consisted in six Petri Dishes (M1-M6), were positioned sufficiently far from the EMF source. It was monitored the temperature and the humidity, which were maintained constant in both irradiated and control lots. Experiment diagram is depicted in the Appendix fig.1 and fig.2. The magnetic induction (B) of the field was measured with a digital teslameter in the indicated points on the drawing. The values are in µT. After germination period (35 days), the plant material was processed to determine the activity of the peroxidase, enzyme involved in the removal of oxidative stress (Artenie et al., 2008). Also it was determined the total protein synthesis and was calculated the percentage number of the germinated seeds. From each sample was counted the number of germinated seeds and reported to the total number of seeds. Data were represented graphically in the diagrams (Appendix at the end of the paper) which appear after the statistical processing. On the charts, the vertical error bars shows the 95% (0.05) confidence level for mean. Interval estimates are often desirable because the estimate of the mean varies from sample to sample. The interval estimate gives an indication of how much uncertainty there is in our estimate of the true mean. The narrower the interval, the more precise is our estimate (Kotz et al., 1988-2008).

RESULTS AND DISCUSSION

Peroxidase, unlike the other two, is an enzyme widespread in all cellular compartments (cell wall, chloroplast, cytosol, endoplasmic reticulum, mitochondria, peroxisome, plasma membrane) (Bakalovic et al, 2006; Passardi et al., 2007; Koua et al., 2008), where the function is to neutralize hydrogen peroxide using various electron donors (Heldt, 2005).

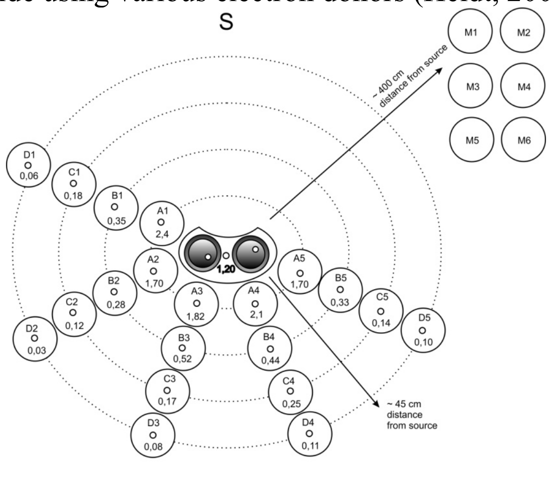

Fig.1. Schematic representation of the experiment. At the center are the two radio stations, around which were arranged the Petri Dishes. In the center of each plate is indicated the magnetic induction in µT.

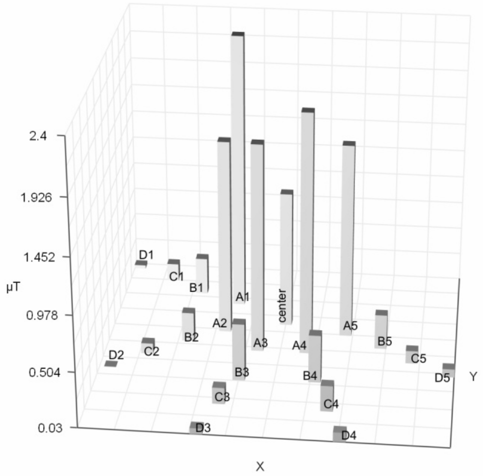

Fig.2. Spatial representation of the intensity of magnetic induction (µT) in relation to the probes arrangement.

Highly reactive oxygen species are generated during aerobic metabolism, and they are normally detoxified by cellular defense mechanisms that involve superoxide dismutase. This enzyme converts superoxide radicals to hydrogen peroxide, which is then converted to water by peroxidase (Taiz and Zeiger, 2002). Peroxidases are heme-containing glycoproteins encoded by a large multigene family in plants. Studies have suggested that peroxidase plays a role in lignification, suberization, auxin catabolism and self-defense against pathogens, salt tolerance and senescence (Atak et al., 2007).

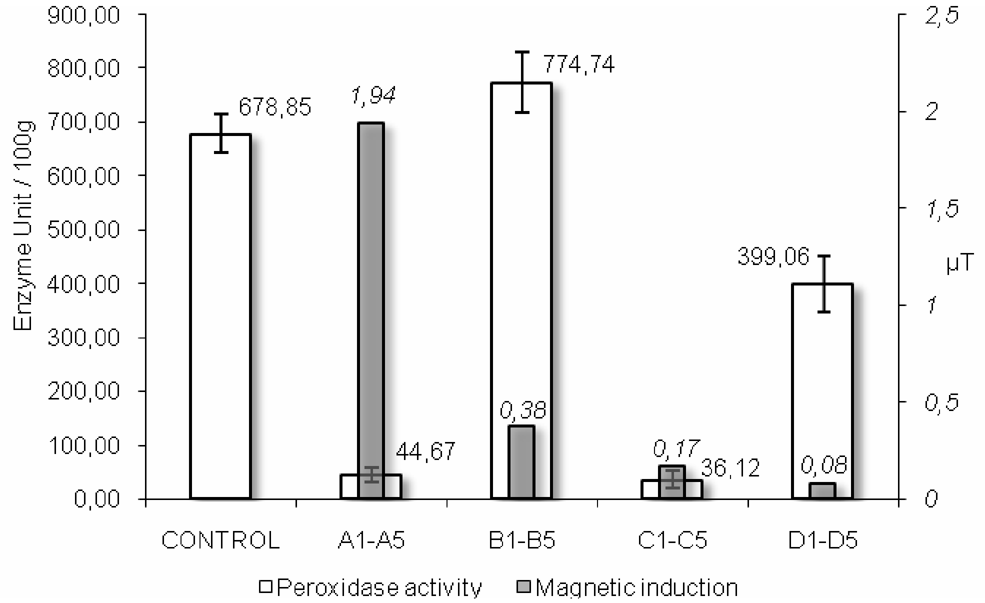

Fig.3. Variation of the peroxidase activity and magnetic induction

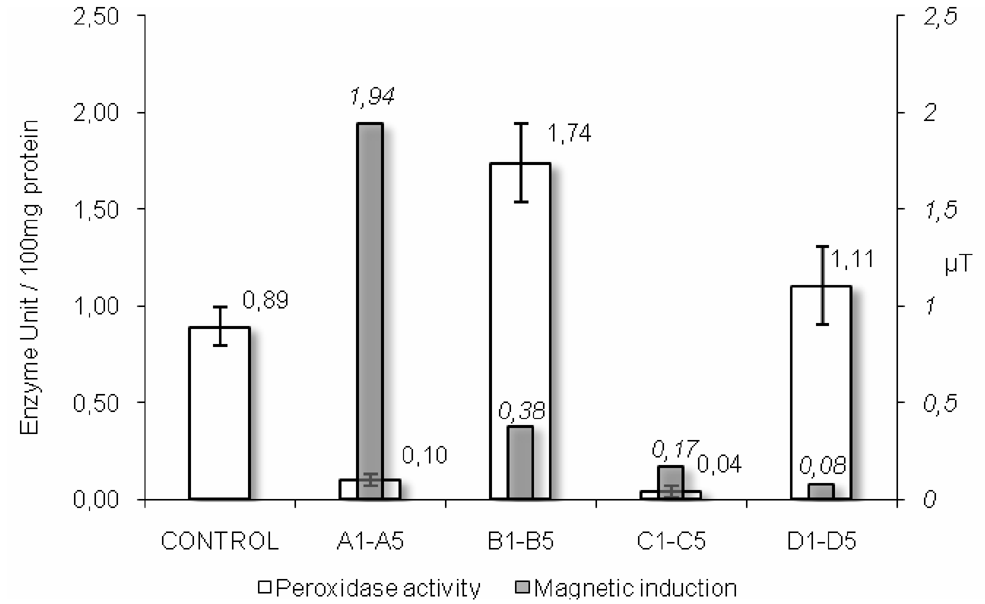

Fig.4. Variation of the peroxidase specific activity and magnetic induction.

The experiment conducted has shown that activity of this enzyme undergoes major fluctuations, as can be seen in fig. 3 and fig. 4. It might say that this pattern of fluctuation could be due to influences exerted by the different intensity of magnetic induction from a cellular compartment to another, which is to have different amounts of enzyme.

The amount of protein highlighted by Bradford method shows a significant variation for A1-A5, B1-B5 and D1-D5 in relation to the control (fig. 5), in such cases were founded decreases. C1-C5 samples, shows an amount of protein approximately equal to control lots. The proteins highlighted in the experiment comes both from the reserve proteins of seeds and "de novo" synthesis.

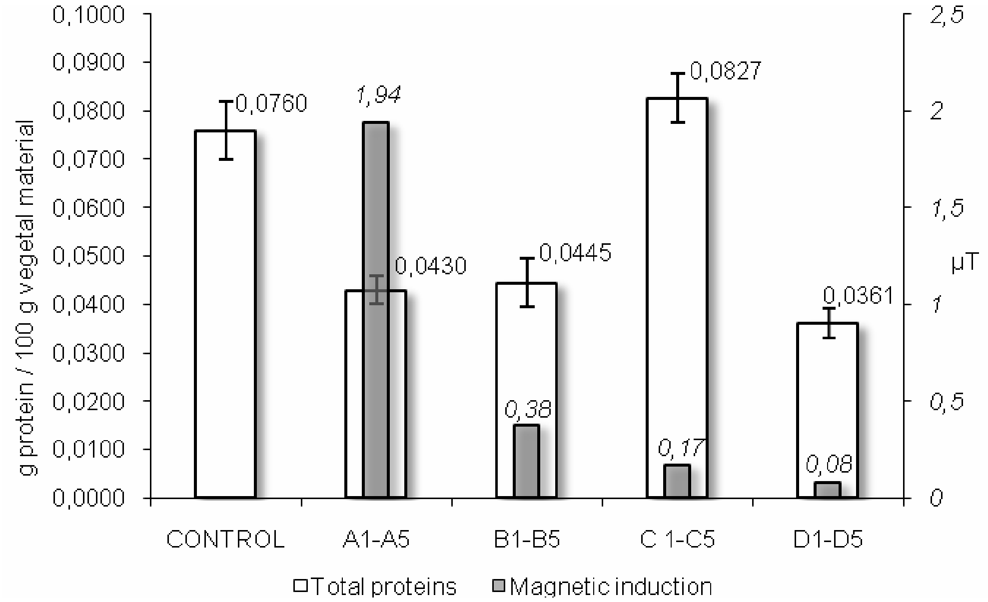

Fig.5. Variation of the total protein quantity and magnetic induction.

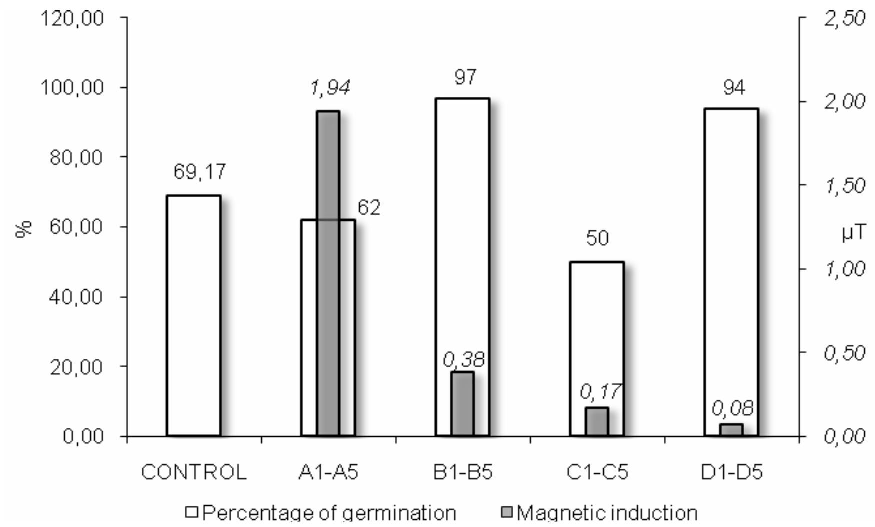

Fig.6. The percentage of seeds germination and the variation of magnetic induction

Percentage of seeds germinated (fig. 6) during the experiment indicates that low intensity magnetic induction can have a stimulating effect. This is observed for samples B1-B5 and D1-D5, where the percentage of germination reached very high values, 97% respectively 94%. In the other two cases, compared with the control, there is a negative trend in germination. In case of A1-A5, high intensity magnetic induction does not seem to have affected germination (percentage difference being only 8% compared to control), where C1-C5 can be considered a decrease of 19%. From germination behavior of toward various intensities of magnetic induction, it may find an indirect correlation between enzyme activities involved in removing the effects of oxidative stress. Since peroxidase takes part to other processes, very high activity in cases B1-B5 and D1-D5 in consistency with high rates of germination may be due to the involvement of this enzyme into other metabolic processes closely related to germination and growth (Atak et al., 2007). From this perspective, the low activities recorded in samples A1-A5 and C1-C5 cannot be attributed to the direct effect of EMF.

CONCLUSIONS

The performed experiment, with 462.6875 MHz electromagnetic radiation frequency, obtained from two emission-reception radio stations, has demonstrated that there is no direct correlation between the intensity of induction and the effects caused by the different magnetic induction during the seeds germination. Thus, there are cases where electromagnetic radiation may be used as a stimulatory agent since two cases were found with a very high percentage of germination in correlation with a high peroxidase activity and low total protein amount. In these circumstances, it is required more detailed investigations, particularly targeted at the induction that caused the stimulation of germination.

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