CAPÍTULO 10

LOW-FREQUENCY ELECTROMAGNETIC FIELDS ELEVATE OXIDATIVE STRESS IN TOBACCO PLANTS

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ABSTRACT: There is growing global interest in the biological effects of non-ionizing electromagnetic radiation, especially extremely low frequency electromagnetic fields (ELF-EMFs) in the environment. While the harmful effects of magnetic fields on humans are yet to be proven, oxidative stress is suggested as a potential cause of adverse effects from ELF-EMFs. This study examined the impact of ELF-EMFs on enzyme activity in tobacco plants under oxidative stress. *Nicotiana tabacum* (L.) seedlings of the Xanthi variety were continuously exposed to 2.0 mT and 60 Hz ELF-EMFs for 24, 48, 72, and 96 hours. The biochemical markers measured included ascorbate peroxidase (APX) and catalase (CAT) activities in leaf and root extracts. Increased APX and CAT enzyme activities were observed in leaf extracts after

48, 72, and 96 hours of magnetic field exposure. Additionally, magnetic field exposure elevated APX enzyme activity in root extracts at 48 and 72 hours, and CAT enzyme activity at 48, 72, and 96 hours. In contrast, 24-hour ELF-EMF exposure did not affect APX and CAT enzyme activities in leaf and root extracts. All treatments were compared with a properly sham-exposed control. These findings suggest that ELF-EMFs induce oxidative stress, potentially enhancing the oxidative defense system in tobacco plants.
KEYWORDS: Electromagnetic fields; Oxidative stress; Ascorbate peroxidase, Catalase, Tobacco plant.

INTRODUCTION

A plethora of studies have been related to the bio-effects of extremely low frequency-electromagnetic fields (ELF-EMFs) on organisms. In most cases, the mechanisms of interaction, which are mainly associated with low and moderate flux density magnetic fields, remain to be elucidated. These mechanisms are mostly related to magnetic interactions with enzyme catalyzed-biochemical processes. Furthermore, a potential link between magnetic fields and their effects on living organisms is the oxidative stress (Zhang et al., 2017; Coballase-Urrutia et al., 2018), for which a potential relationship between exposure to magnetic fields and antioxidant systems has observed (Scaiano et al., 1994; Li and Chow, 2001; Kula et al., 2002; Zhang et al., 2003).

Regarding plants, the effects of ELF-EMFs and static magnetic fields (SMFs) exposure on oxidative stress in plants has been widely investigated (Tkalec et al., 2007; Sharma et al., 2009; Nabizadeh et al. 2014; Jouni et al., 2012), indicating an increased oxidative enzyme activity as a consequence of magnetic field exposure.

In a long-term exposure experiment, Mahmood et al. (2013) showed that peroxidase enzyme activity of oil palm exposed for six months or seven years to electromagnetic fields from a 275 KV high-voltage transmission line was significantly increased, suggesting an oxidative stress response.

On the other hand, tobacco plant offers a versatile model to evaluate physiological changes induced for a variety of factors (Ganapathi et al., 2004), for which we selected this plant model to evaluate EMFs effects on enzyme activities of oxidative stress. For instance, Sahebjamei et al. (2007) investigated the effects of magnetic fields at 10 mT and 30 mT on the antioxidant enzyme activities of suspension-cultured tobacco cells. They found an increased superoxide dismutase activity and decreased catalase and ascorbate peroxidase activities induced by SMF exposure. Furthermore, the level of lipid peroxidation was also increased by magnetic fields, thus indicating that SMFs may deteriorate antioxidant defense system of plant cells. Moreover, Touati et al. (2013) observed that SMFs at 100 mT exposure treatment on *Raphanus sativus*, caused a significant increase of catalase activity in radish plantlets.

Because of magnetic fields are ubiquitous abiotic stressors, they affect life bodies in general. Previous research has reported the effects of EMFs on a variety of agriculturally important plants (Angel et al., 2005). Based in the increasing research regarding this concern, it has been proposed that oxidative stress underlies EMFs effects on plants (Tkalec et al.2005; Gill and Tuteja, 2010; Serdyukov and Novitskii, 2013). On the other hand, science faces the serious challenge of doubling global crop production by 2050, due to the continuing increase in world population and to meet future food demand, changes in diet and increased consumption of biofuels (Ray et al., 2013). To solve this problem, research is carried out on different topics in agronomy, one of these is the study of the effects of applying electromagnetic fields to plants, which have shown several effects on growth (Pietruszewski and Martínez, 2015).

Taken together, we have undertaken the present study to further evaluate the effect of ELF-EMFs on enzyme activity of oxidative stress in tobacco plants.

MATERIALS AND METHODS

Plant material and growth conditions

Tobacco seeds (Xanthi variety of *Nicotiana tabacum* L.) were obtained from the Centro de Investigación y Estudios Avanzados at Instituto Politécnico Nacional, Unidad Irapuato, Guanajuato, México. They were soaked in 70% ethanol for one minute, 20% sodium hypochlorite solution for 20 min, and rinsed with sterile distilled water for 30 min, after which they were planted in Petri dishes (15 seeds/dish), containing MS culture medium (Murashige and Skoog, 1962) and allocated in an environmental chamber (Biotronette Mark III, Lab-Line Instruments, Melrose Park, IL) maintained at 25 ± 0.5 °C undisturbed. We selected seedlings with straight primary roots (21 days aged) for the bioassays and used 15 plantlets for each studied group. Furthermore, to determine the viability of the tobacco seeds, a tetrazolium test was carried out prior to germination experiments. The seeds were soaked in distilled water for 24 hours, then the water was removed and a 1.00% tetrazolium solution was added, covering all the seeds. The seeds were incubated for 3 days at room temperature and protected from light. After this incubation period, the topographic staining pattern was demonstrated with aid of a stereoscopic microscope and the viability percentage was estimated (95% seed-viability was observed). ELF-EMFs treatment regimens are detailed below.

Magnetic field exposure facilities and measurements

We used a standardized and calibrated home-made magnetic field exposure facility, as previously reported by our research team (Heredia-Rojas et al., 2004; Heredia-Rojas et al., 2010; Heredia-Rojas et al., 2018). In brief, a coil was prepared by winding 552 turns of enamel-insulated copper wire (1.3 mm diameter), which formed a 13.5-cm radius and 71-cm length cylindrical solenoid and was connected to step-down and variable transformers, plugged to a 110 V AC source. Cultured seedlings were placed in the middle of this structure in predetermined zones, where the magnetic field was homogeneous with rms value of 2.0 mT, and kept at 25 °C ± 0.2 °C and 45% humidity. Sham-treated seedlings were used as negative controls, which were placed in the same room in a magnetic field device of identical characteristics turned off.

Magnetic flux density was determined by a Gaussmeter (Bell FW 6010, Orlando, FL) and an attached oscilloscope (BK-Precision model 2120; Dynascan Corp., Chicago, IL), which was required to monitor the resulting field and a 60-Hz alternating sinusoidal magnetic field was generated. Frequency content was almost pure 60 Hz (< 2% total harmonic distortion) and 0.3 μT and 20 μT values were observed for respective background magnetic and local geomagnetic fields.

Experimental protocol

In the present study, we performed the experiments with tobacco seedlings in the presence or absence (sham) of continuous exposure of ELF-EMFs, at 60 Hz frequency and 2.0 mT of magnetic flux density, for 24 h, 48 h, 72 h, and 96 h. We selected 15 seedlings to measure APX and CAT enzyme activities in root and leaf extracts.

Extraction and assay of the enzymes of antioxidant system

In summary, samples of 200 mg (fresh weight) of leaves and roots were homogenized separately in 1.0 ml of 50 mM Na-phosphate buffer (pH 7.0) containing 5 mM ascorbate, 5 mM dithiothreitol (DTT), 5 mM EDTA,100 mM NaCl, and 2% (w/v) polyvinylpyrrolidone (PVP). The homogenate was filtered through sterile gauze and then centrifuged at 15,000g for 15 min at 4°C. The supernatant was recovered which was used for the enzymatic analysis. Protein contents were determined by the spectrophotometric method of Bradford (1976) at 280 nm, using bovine serum albumin (BSA) as standard.

The APX activity was determined in accordance to the method described by Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mm EDTA, 5 mM ASH and 10 μl enzyme aliquot in 200 μl final volume. The reaction was started by adding H_2O_2 to a final concentration of 1 mM. The reaction rate was monitored by the decrease in absorbance at

290 nm. The rate constant was calculated using the ASH extinction coefficient of per min-1 mg-1 of protein present and corrected for the rate obtained prior to the addition of H_2O_2. APX activity was expressed as units of enzyme activity (U), where 1 U is the amount of enzyme in mg that is needed to oxidize 1 μmol of ascorbic acid to ascorbate during 1 min.

The CAT activity was measured according to the original method proposed by Beers and Sizer (1952). The reaction mixture contained 50 mM Potassium Phosphate Buffer pH 7.0 and 10 μl enzyme aliquot in 200 μl final volume. The reaction was started by adding H_2O_2 to a final concentration of 5 mM. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. CAT activity was expressed as units of enzyme activity (U), where 1 U is the amount of enzyme in mg that is needed to oxidize 1 μmol of H_2O_2 during 1 min.

Statistical analysis

Statistical differences for APX and CAT activities between treated cultures and sham-exposed controls were calculated by a *t*-test for independent samples. Data normality was calculated by the Kolmogorov-Smirnov test ($p < 0.05$). All analyses were performed using the SPSS software version 20.0 (SPSS Inc., Chicago, IL).

RESULTS

Figure 1 shows the APX activity in tobacco seedlings leaf extracts. A significant ($p < 0.05$) increase in APX activity was observed at 48 h, 72 h, and 96 h of continuous ELF-EMFs exposure at 2.0 mT and 60 Hz frequency, as compared with sham-exposed controls ($p < 0.05$), whereas Figure 2 depicts APX activity values for root extracts. The APX activity was also significantly ($p < 0.05$) increased at 48 h and 72 h exposure to ELF-EMFs but no difference was found for 24 h and 96 h exposure conditions, as compared with sham-exposed cultures ($p > 0.05$).

In addition, Figure 3 shows CAT´s activity values in leaf extracts after exposure of tobacco plantlets at 2.0 mT and 60 Hz magnetic fields. A statistically significant ($p < 0.05$) increase in CAT activity was observed at 48 h, 72 h, and 96 h exposure to ELF-EMFs ($p < 0.05$). However, we did not find differences at 24 h continuous exposure, as compared with sham-exposed control cultures ($p > 0.05$). In Figure 4, we showed CAT activity values for root extracts. The level of CAT activity was also modified after magnetic field exposure and we observed an increased activity at 48 h, 72 h, and 96 h exposure to ELF-EMFs ($p < 0.05$). However, no significant changes were observed at 24 h exposure compared with sham-exposed controls ($p > 0.05$).

DISCUSSION

We evaluated the effect of ELF-EMFs at 60 Hz of frequency and 2.0 mT of magnetic flux density on oxidative stress enzyme activities on tobacco plants. Results showed that magnetic fields increased APX and CAT activities in leaf and root extracts after 48 h, 72 h, and 96 h of magnetic field exposure. These findings evidenced a plant response against oxidative stress caused by magnetic field exposure, which is critical in part, to understand the mode of action of magnetic fields on life bodies. Since the 1970s, oxidative stress has been recognized as an inducer of cytotoxicity. Because of this, a plethora of studies have reported protective benefits of antioxidants (Schmidt et al., 2015; Xu et al., 2017).

As magnetic fields are widely distributed in the modern environment, they may affect organisms, thus increasing the concern about the possible risk of functional disorders due to exposure to this non-ionizing radiation, particularly in plants, which do not escape from ELF-EMFs sources. A general hypothesis claims that magnetic fields, as an abiotic stressor, are associated with oxidative stress, which in turn, increases activity, concentration, and lifetime of free radicals, in particular, reactive oxygen species (ROS) (Allen,1995; Mittler,2002). It is well known that ROS are produced within cells as a consequence of normal metabolic processes, however under stress conditions, ROS production increases (Smirnoff, 1993). Thus, abiotic stress results in the formation of ROS in plants that makes the condition called oxidative stress (Apel and Hirt, 2004). Under stress conditions, similar to ELF-EMF exposure used in the current study, we hypothesized that the generation of ROS is unavoidable and plants need to have a set of enzymatic antioxidant molecules to protect themselves from cellular damage, as previously reported (Foyer and Noctor, 2000; Burritt and MacKenzie, 2003), which agrees with our results, indicating an elevation of oxidative stress enzyme activity.

Recently, Kthiri et al. (2019) demonstrated that an exposure to SMFs at 250 mT on *Saccharomyces cerevisiae* cultures, modified the activities of catalase, superoxide dismutase, and glutathione peroxidase, suggesting an oxidative stress. In this regard, Kıvrak et al. (2017) observed that ELF-EMFs exposure negatively affected the antioxidant defense system, which become relevant considering that oxidative stress occurs when antioxidant defense system does not prevent the harmful effects of free radicals. Moreover, it has been proposed that DNA is indirectly affected by the action of ELF-EMFs, due to anomalous movement of electrons (Valberg et al., 1997), which may generate guanine radicals, that in turn, upon reaction with water may induce oxidative DNA damage (Giese, 2006). In a previous report (Heredia-Rojas et al., 2020), we demonstrated that ELF-EMFs clastogenic effect induced in mouse bone marrow was significantly reduced, when animals were pretreated with the widely used antioxidant resveratrol, using ELF-EMFs exposure of similar characteristics of those used here. These findings suggest the involvement of an oxidative stress in magnetic fields-induced clastogenicity.

In contrast, several reports have claimed an anti-oxidative effect of magnetic fields, particularly ELF-EMFs similar of those used in the current study but applied on mammalian cells (Balind et al., 2014; Cichoń et al., 2017). More recently, Ahn et al. (2020) observed that an exposure to pulsed magnetic fields (PMFs) prevented red blood cells from oxidative stress. Furthermore, with regard to mammalian cells, it has been proposed that magnetic field exposures are closely related with an increase in the activity, concentration, and lifetime of free radicals after SMFs (Politański et al. 2010; Ghodbane et al., 2013) or electromagnetic field exposure (Kerimoğlu et al., 2018).

In conclusion, ELF-EMFs at 2.0 mT and 60 Hz exposure induced an oxidative stress, and as a consequence, potenciated the oxidative defense system in tobacco plants. Despite the mechanism of action for cytotoxicity induced by ELF-EMFs has not yet been elucidated, reports have shown intrinsic electrical features associated with biostructures and biological functions such as development, growth, and repair. Furthermore, living organisms are sensitive to external electromagnetic fields of very week intensities, which may be debatable. The lack of realistic mechanisms to couple exposure to ELF-EMFs and biological events has resulted in many unfocused investigations, inconsistent observations, and interpretations. Nowadays, there is no accepted mechanism by which ELF-EMFs consistently produced an oxidative stress condition. However, most researchers agree that oxidative stress is one of the main biological magnetic field effects. Further studies are required to fully understand this phenomenon.

AUTHOR´S CONTRIBUTIONS

J. Antonio Heredia-Rojas**:** Design of the experiments, bioassays, experimental procedures; Abraham O. Rodríguez-De la Fuente: Conceptualization, data curation and statistical analyses; Erick Freeze-Gallardo: Experimental procedures; Deyanira Quistian-Martínez: Supervision, writing-review and editing; Ricardo Gomez-Flores: Substantially revised the work and original draft preparation; David F. La fuente-Rincón: Experimental procedures, supervision; Omar Heredia-Rodríguez: Magnetic field exposure facilities and measurements and Alberto Valadez-Lira: Critically revising the manuscript and original draft. All authors read and approved the final manuscript.

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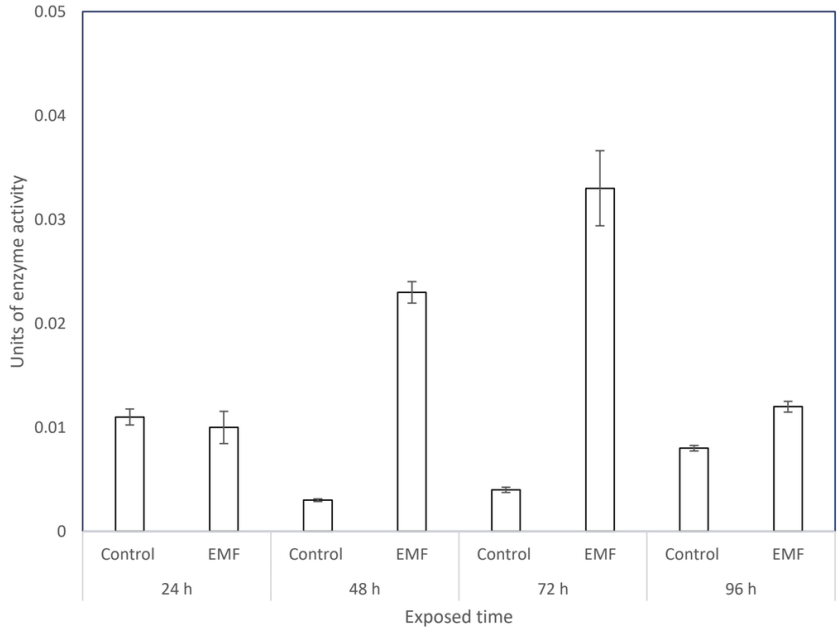

Figure 1. Effect of ELF-EMFs on APX activity in leaf extracts of tobacco seedlings. A significant increased APX activity was observed at 48 h, 72 h, and 96 h of continuous exposure of ELF-EMFs at 60 Hz and 2.0 mT, as compared with sham-exposed controls ($p < 0.05$). Bars represent grouped arithmetical means ± standard error.

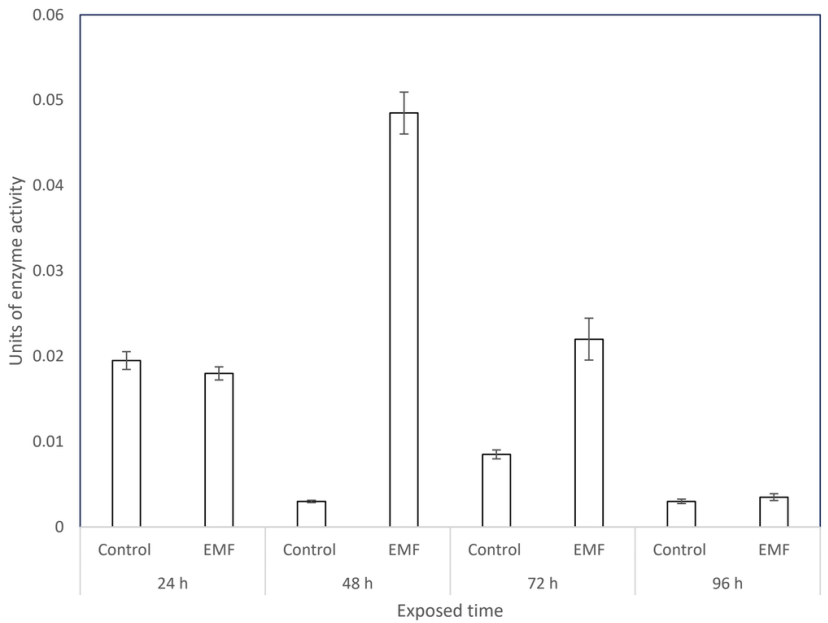

Figure 2. Effect of ELF-EMFs on APX activity values in root extracts of tobacco seedlings. APX activity increased at 48 h and 72 h of continuous exposure of ELF-EMFs at 60 Hz and 2.0 mT ($p < 0.05$) but no difference was found for 24 h and 96 h exposure conditions, as compared with sham-exposed cultures. Bars represent grouped arithmetical means ± standard error.

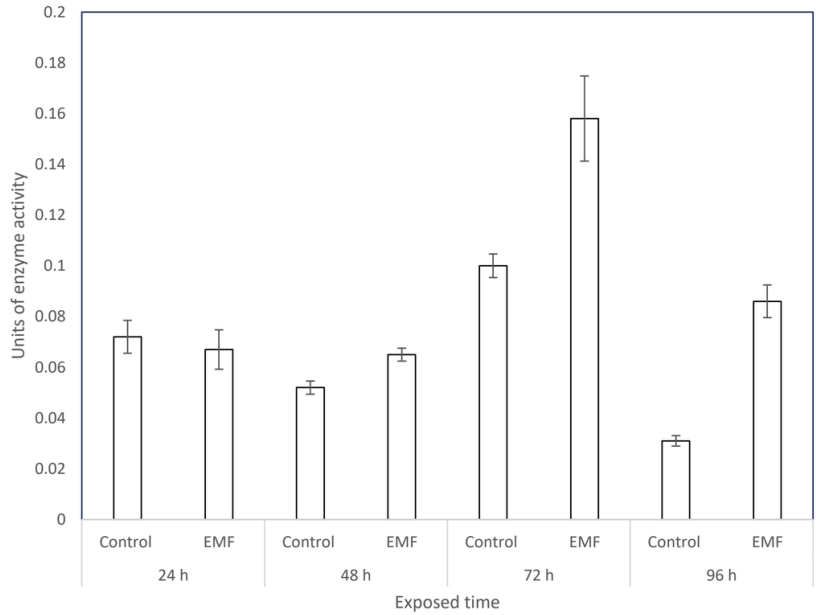

Figure 3. Effect of ELF-EMFs on the CAT enzyme activity in leaf extracts of tobacco seedlings. A statistically ($p < 0.05$) significant increase in CAT activity was observed at 48 h, 72 h, and 96 h of ELF-EMFs exposure. We did not observe differences at 24 h of ELF-EMFs exposure, as compared with control cultures. Bars represent grouped arithmetical means ± standard error.

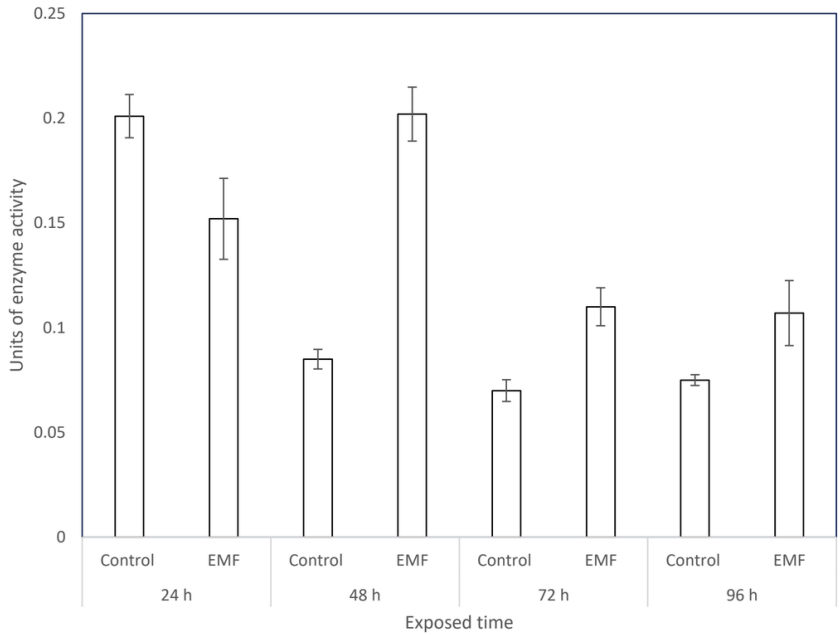

Figure 4. Effect of ELF-EMFs on the CAT enzyme activity in root extracts of tobacco seedlings. A statistically ($p < 0.05$) significant increase in CAT activity was observed at 48 h, 72 h, and 96 h of ELF-EMFs exposure. We did not observe differences at 24 h of ELF-EMFs exposure, as compared with control cultures. Bars represent grouped arithmetical means ± standard error.