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ALTERATIONS IN ENZYME ACTIVITIES IN LEAVES AFTER EXPOSURE OF *PLECTRANTHUS SP.* PLANTS TO 900 MHZ ELECTROMAGNETIC FIELD

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ABSTRACT

Accelerated and widespread use of different communication systems and modern electronic equipment increases exposure to radiofrequency electromagnetic fields (RF EMF) and raises serious concerns about the biological and health-related effects of RF radiation. Numerous studies document various biological effects of RF radiation. However, most of these findings derive from epidemiological, animal and in vitro studies while only a few investigate effects of RF radiation on plants.

*The purpose of our study was to investigate the alterations in enzyme activities in leaves after exposure of plants *Plectranthus sp.* to 900 MHz EMF and their dependence on the time elapsed after exposure.*

*Plectranthus is the largest South African genus of plants belonging to the mint family (Lamiaceae). The ornamental herbaceous plant *Plectranthus sp.* was used as a model plant for investigating the effects of 900 MHz EMF. Plants were exposed whole body for 1 hour to EM radiation from GSM mobile phone (carrier frequency 902 MHz, 2 W pulse output power) at 20 cm distance from the antenna. Alterations in activity of isocitrate dehydrogenase, malate dehydrogenase and glucose-6-phosphate dehydrogenase in leaves were registered immediately after the end of the exposure and 1, 2 and 24 hours later. Irradiation of plants induced different alterations in enzyme activities depending on the time elapsed after irradiation. Immediately after exposure the activity of the three investigated enzymes decreased, but increased at 24th hour.*

In conclusion, the data provide evidence that plants perceive and respond to electromagnetic fields and are a good model to study the effects of mobile phone radiation.

Keyword: enzyme activity, 900 MHz electromagnetic field, mobile phone, plant leaves

Introduction

Accelerated and widespread use of different communication systems and modern electronic equipment has increased exposure to radio frequency electromagnetic fields (RF EMF) and has raised serious concerns about the biological and health-related effects of RF radiation. Numerous studies have documented various biological effects of RF radiation. Most of them concern health, brain and DNA effects. However, most of these findings derived from epidemiological, animal and *in vitro* studies while only a few investigated effects of RF radiation on plants. Assessment of the RF EMF impact on plants is of great importance because plants have an important role in the living world as main primary producers

of organic compounds and oxygen.

Plants are continuously exposed to various environmental stresses and display a wide spectrum of developmental and biochemical responses contributing to stress adaptation. Their physiological responses can be evoked by a great variety of external stimuli, including mobile phone radiation.

Some authors investigated responses of tomato plants (*Lycopersicon esculentum* Mill. VFN8) to exposure to low level 900 MHz EMF for 10 min and provide evidence that they perceive and respond to EMF as an injurious treatment (wound). High frequency EMF is an environmental stimulus that affects gene expression, transcription, translation and calcium and energy charge in tomato plants (2; 14; 19). Tkalec et al. investigated the effects of exposure of the duckweed *Lemna minor* to RF EMFs (400 MHz, 900 MHz and 1.9 GHz, different electric field strengths and exposure

durations (2, 4 and 14 h)) on plant growth and parameters of oxidative stress. The data suggest that non-thermal exposure to investigated RF EMFs, especially at 900 MHz, induced oxidative stress in duckweed and might influence plant growth. Observed responses depended on the field frequency as well as other exposure parameters like field strength, modulation and exposure time (17; 18). Schmutz et al. investigated the long-term effect of 2.45 GHz radiation on trees spruce (*Picea abies* (L.) Karst.) and beech (*Fagus sylvatica* L.). EM radiation caused no visual symptoms of damage. The only effect of microwave exposure was a decrease of calcium and sulfur concentrations in beech leaves with increasing power flux densities (15). Tafforeau et al. studied the effects of 100 GHz EM radiation on flax (*Linum usitatissimum* L. var Ariane). A single 2 h exposure to 105 GHz at non-thermal levels induces meristem production with kinetics similar to that induced by weak environmental stimuli and irradiation from GSM telephone (16). Magone used a sensitive clone of *Spirodela polyrhiza* (L.) Schleiden to study the effects of the Skrunda Radio Location Station in Latvia (156–162 MHz, 5 days exposure). The vegetative reproduction rate was accelerated in the first 20 days after the end of exposure. The vegetative growth rate was lower in plants just beginning formation. At 55 days, various morphological and developmental abnormalities appeared. Plants developed completely to daughter fronds under EMF exposure had a shorter life-span and a fewer subsequent daughters (11).

There still are not enough investigations and sufficient explanation for the mechanisms of observed biological effects of radiofrequency EMF, particularly in the absence of gross cell/tissue heating effects. Investigation of RF EMF effects on enzymes would contribute to clarifying mechanisms of effects of this radiation on organisms. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell. Consequently, the network of metabolic pathways within each cell depends on the set of functional enzymes that are present. The limited number of studies on enzyme systems has yielded mixed results. Enzyme activity was increased, decreased or did not altered depending on the exposure parameters (frequency, modulation, exposure duration, SAR), enzyme type and localization (1; 5; 4; 6; 13). Some authors investigated antioxidant enzymes (7; 8; 12). The question of

whether the radiofrequency fields of cellular phones affect oxidative stress and the antioxidase activities are of considerable interest to biomagnetics and biochemistry.

The purpose of our study was to investigate the alterations in enzyme activities in leaves after exposure of plants *Plectranthus* sp. to 900 MHz EMF and their dependence on the time elapsed after exposure.

Materials and methods

Plectranthus is the largest South African genus of plants belonging to the mint family (*Lamiaceae*). There are many *Plectranthus* species (around 44) that are currently used as ornamental herbaceous plants throughout the world's gardens. Several species are grown as leaf vegetables, as root vegetables for their edible tubers, or as medicine. *Plectranthus* species are used as food plants by the larvae of some *Lepidoptera* species.

Plants *Plectranthus* sp. were grown in a growing chamber at controlled temperature (23–24°C), light (30–40 mmol m⁻²s⁻¹), humidity (60–65%) and photoperiod 12/12 h.

The exposure was performed by GSM mobile phone type NSM-3, which was connected to a personal computer through an interface cable. The mobile phone was programmed via special software to emit carrier frequency 902 MHz (60th uplink channel of GSM900). The signal included standard GSM modulation. GSM signal was pulsed. The duration of the pulses was 577 microseconds that corresponded to one time slot of TDMA frame. The TDMA frame in GSM systems has a duration 4.615 milliseconds. Discontinuous transmission mode (DTX) was off during all exposures. Mobile phone pulse output power was 2 W. Plants were exposed whole body to EM radiation from GSM mobile phone at 20 cm distance from the antenna for 1 hour. Alterations in enzyme activity in leaves were registered immediately after the end of the exposure and 1, 2 and 24 hours later. At the same time control pots were kept at the same conditions, but without exposure.

Glucose-6-phosphate dehydrogenase activity was determined according to the method of Wakiuchi et al. (20). This method, as well as the methods for determination of NADH-malate dehydrogenase and NADP-isocitrate dehydrogenase activities are described in Handbook of Plant Physiology (9). Protein quantity was determined according to the method of Bradford (3).

The presented data are averages and standard deviations of 6 measurements for each term. The Student *t*-test was used to estimate the statistically significant differences between

the experimental samples.

Results and Discussion

The citric acid cycle (tricarboxylic acid cycle, Krebs cycle), is a series of enzyme-catalysed chemical reactions of central importance in all living cells that use oxygen as part of cellular respiration. In eukaryotes, the citric acid cycle occurs in the matrix of the mitochondrion. It is part of a metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate a form of usable energy.

Nicotinamide adenine nucleotide (NADH)-dependent malate dehydrogenase (MDH, EC 1.1.1.37) is an enzyme in the citric acid cycle that catalyzes the conversion of malate into oxaloacetate (utilizing the NAD/NADH cofactor system) and vice versa (reaction is reversible): (S)-malate + NAD⁺ ↔ oxaloacetate + NADH + H⁺. The enzyme exists in all aerobics organisms. There are two MDH isozymes in eukaryotic cells: one which is located in the mitochondrial matrix and the other in the cytoplasm. In the plant's chloroplast there is an additional NADP-dependent form of MDH (EC 1.1.1.82).

Exposure of *Plectranthus* plants to 900 MHz for 1 hour EMF leads to decrease of MDH activity immediately after the end of treatment, as well as 2 hours later. MDH activity highly increases at 24th hour after exposure (Fig. 1).

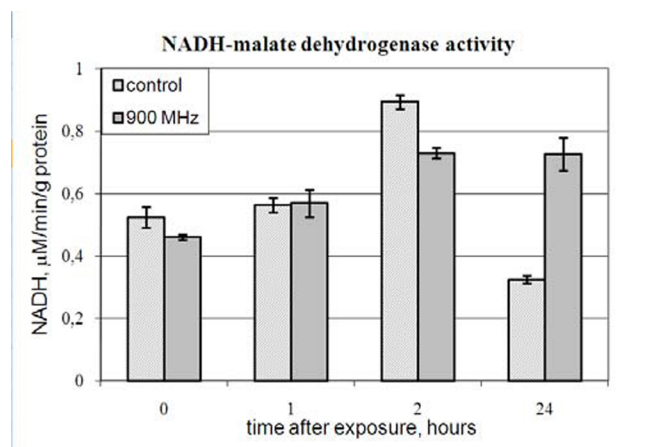


Fig. 1. Alterations in NADH-MDH activity in *Plectranthus* leaves after 1 hour exposure to 900 MHz radiation.

Isocitrate dehydrogenase (ICDH) is also an enzyme in the citric acid cycle. In eukaryotes, ICDH exists in two forms: an NAD⁺-linked enzyme (EC 1.1.1.41) found only in mitochondria and a NADP⁺-linked enzyme (EC 1.1.1.42) that is found in both mitochondria and cytoplasm. The ICDH step is one of the irreversible reactions in the citric acid cycle and

therefore must be carefully regulated to avoid unnecessary depletion of isocitrate (and therefore an accumulation of alpha-ketoglutarate (α-KG)). In higher plants, α-KG provides the primary carbon skeleton for the assimilation of reduced inorganic nitrogen into amino acids through the glutamine synthetase/glutamate synthase cycle, and thus represents a key point in the linking of carbon and nitrogen metabolism. Plant NADP-ICDH is predominantly localized in the cytosol. Cytosolic NADP-ICDH is the major catalyst for the production of α - KG required for nitrogen assimilation. It is the predominant form in leaf extracts.

NADPH is an essential electron donor in numerous biosynthetic and detoxification reactions. In animal, yeast and bacteria, the NADP-ICDH, which catalyzes the production of NADPH, is being recognized as an essential component of the antioxidative defence mechanisms. In plant cells, there is little information on the antioxidant properties of NADP-ICDH. The data obtained from Leterrier et al. indicate that in pea plants the cytosolic NADP-ICDH shows a differential response, at mRNA and activity level, depending on the type of abiotic stress and suggests that this dehydrogenase could have a protective antioxidant role against certain environmental stresses in plants (10).

Exposure of *Plectranthus* plants to 900 MHz EMF for 1 hour decreases NADP-ICDH activity for one hour after the treatment (Fig. 2). Two hours later ICDH activity increases and remains increased up to 24th hour after exposure

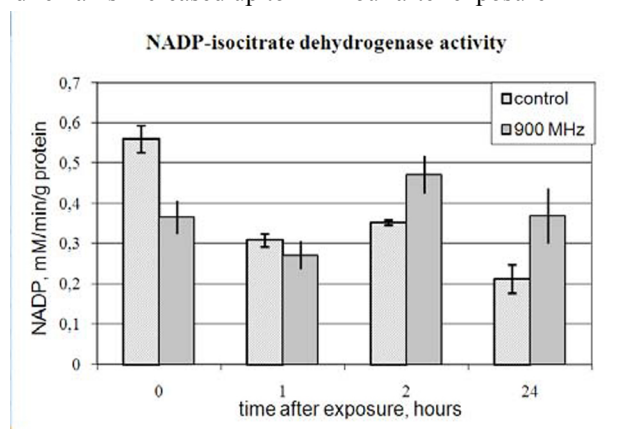


Fig. 2. Alterations in NADP-ICDH activity in *Plectranthus* leaves after 1 hour exposure to 900 MHz radiation.

The pentose phosphate pathway (PPP) is a cytosolic metabolic pathway that serves to generate NADPH and the synthesis of pentose (5-carbon) sugars and supplies reducing energy to cells. This pathway is an alternative to glycolysis. Glucose-6-phosphate dehydrogenase (G6PD) is a cytosolic enzyme in the PPP. In higher plants, several isoforms of

G6PDH have been reported, which are localized in the cytosol, the plastidic stroma, and peroxisomes.

G6PDH, the rate-limiting enzyme of PPP, determines the amount of NADPH by controlling the metabolism of glucose via the PPP. It is found that G6PDH played a role in against oxidative stress in human erythrocytes that lack any other NADPH-producing route. Recent results have demonstrated that this enzyme also plays a protective role against ROS in nucleated eucaryotic cells that possess alternative routes for the production of NADPH. Using G6PDH-deficient cell lines it is showed that other sources of NADPH do not adequately replace the lack of NADPH production by G6PDH. That is, the G6PDH-deficient cells were highly sensitive to oxidative stress compared with cells expressing endogenous levels of G6PDH. Furthermore, human hepatoma cells and yeast containing high expression of G6PDH are resistant to oxidative stress. In animal cells, G6PDH expression is enhanced by oxidative stress by induced drugs that either increase the intracellular concentration of active oxygen species (AOS) or decrease the glutathione (GSH) pool. Yu et al. showed that G6PDH activation is inversely correlated to GSH intracellular levels. The cellular GSH or GSSG possess roles in the regulation of defense-related gene expression in plant cells. The simultaneous increase in G6PDH activity resulted from decreased GSH pool rather than AOS production (21).

Exposure of *Plectranthus* plants to 900 MHz EMF for 1 hour decreases G6PDH activity immediately after exposure and 2 hours later (Fig.3). It is higher at 1st and 24th hour after the treatment.

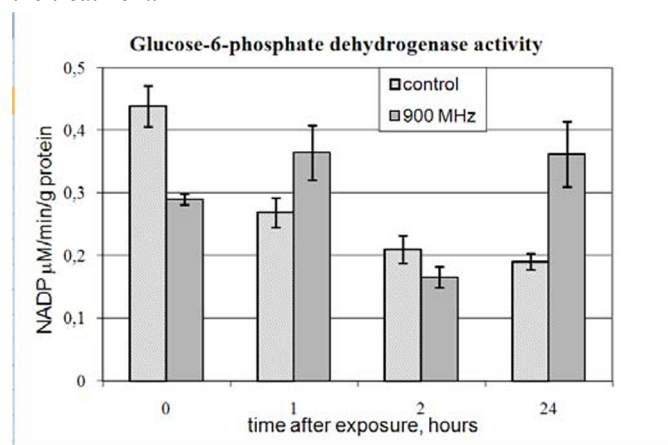


Fig. 3. Alterations in G6PDH activity in *Plectranthus* leaves after 1 hour exposure to 900 MHz radiation.

The activities of enzymes could change by various stress

conditions. Irradiation of plants to 900 MHz EMF for one hour induced different alterations in enzyme activity depending on the time elapsed after irradiation. Immediately after exposure the activity of the three investigated enzymes (NADH-MDH, NADP-ICDH and G6PDH) decreased, but increased at 24th hour after exposure. Alterations in the activities of these enzymes mean alterations in respiration and energy supply of cells and in case of alterations in NADP-ICDH and G6PDH activity – impact on antioxidant defence of organisms.

In conclusion, the data provide evidence that plants perceive and respond to radiofrequency electromagnetic fields and are a good model to study the effects of mobile phone radiation.

REFERENCES

1. **Ammaria M., Lecomte A., Sakly M., Abdelmelek H., de-Seze R.** (2008) *Toxicology*, **250**:70–74.
2. **Beaubois E., Girard S., Lallechere S., Davies E., Paladian F., Bonnet P., Ledoigt G., Vian A.** (2007) *Plant Cell Environ*; **30**(7):834–844.
3. **Bradford M.** (1976) *Anal Biochem*, **72**:248–254.
4. **Dolgacheva L.P., Semenova T.P., Abzhalelov B.B., Akoev I.G.** (2000) *Radiats Biol Radioecol.*, **40**(4):429–32.
5. **Eraslan G., Bilgili A., Akdogan M., Yarsan E., Essiz D., Altintas L.** (2007) *Ecotoxicology and Environmental Safety*, **66**:287–289.
6. **Galvin M.J., Parks D.L., McRee D.I.** (1981) *Radiat Environ Biophys*; **19**(2):149–156.
7. **Höytö A., Juutilainen J., Naarala J.** (2007) *Int J Radiat Biol*; **83**(6): 367–374.
8. **Irmak M.K., Fadillioglu E., Gulec M., Erdogan H., Yagmurca M., Akyol O.** (2002) *Cell Biochem Funct*; **20**(4):279–283.
9. **Kimenov G., Kovacheva N., Tzoneva P., Uzunova A., Gemishev Tz., Andreev G., Peicheva S.** (1995) *Handbook of Plant Physiology*, University Press, Sofia, p.242 (in Bulgarian).
10. **Leterrier M., Del Río L.A., Corpas F.J.** (2007) *Free Radic Res*, **41**(2):191–199.
11. **Magone I.** (1996) *Sci Total Environ*, **180**(1):75–80.
12. **Moustafa Y.M., Moustafa R.M., Belacy A., Abou-El-Ela S.H., Ali F.M.** (2001) *Journal of Pharmaceutical and Biomedical Analysis*, **26**:605–608.

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13. **Paulraj R., Behari J.** (2004) *Mutat Res.*, **545(1–2)**:127–130.
 14. **Roux D., Vian A., Girard S., Bonnet P., Paladian F., Davies E., Ledoigt G.** (2008) *Planta*, **227(4)**:883–891.
 15. **Schmutz P., Siegenthaler J., Staeger C., Tarjan D., Bucher J.B.** (1996) *Sci Total Environ*, **180(1)**:43–48.
 16. **Tafforeau M., Verdus M.C., Norris V., White G.J., Cole M., Demarty M., Thellier M., Ripoll C.** (2004) *Bioelectromagnetics*; **25(6)**:403–407.
 17. **Tkalec M., Malaric K., Pevalek-Kozlina B.** (2005) *Bioelectromagnetics*, **26 (3)**:185–193.
 18. **Tkalec M., Malarić K., Pevalek-Kozlina B.** (2007) *Science of the Total Environment*, **388**:78–89.
 19. **Vian A., Roux D., Girard S., Bonnet P., Paladian F., Davies E., Ledoigt G.** (2006) *Plant Sign Behav*; **1(2)**:67–70.
 20. **Wakiuchi N., Matsumoto H., Takashi E.** (1971) *Phisiol Plant*, **24**:248–253.
 21. **Yu L-J., Lan W.Z., Chen C., Yang Y.** (2004) *Plant Science*, **167**:329–335.