PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 69 (2015), No. 3 (696), pp. 82–86.
DOI: 10.1515/prolas-2015-0012

COMBINED EFFECTS OF 50 HZ ELECTROMAGNETIC FIELD AND SIO_2 NANOPARTICLES ON OXIDATIVE STRESS IN PLANT'S GAMETIC CELLS

Dace Grauda[1#], Anton Kolodynski[1], Inta Belogrudova[1], Lada Bumbure[2], and Isaak Rashal[1]

[1] Institute of Biology, University of Latvia, Miera iela 3, Salaspils, LV-2169, LATVIA

[2] Biomedical Engineering and Nanotechnology Institute, Rīga Technical University, Kaļķu iela 1, Rīga, LV-1658, LATVIA

Corresponding author; dace@email.lubi.edu.lv

Contributed by Isaak Rashal

The paper presents the results of combined effects of an extremely low frequency electromagnetic field (50 Hz) (ELF EMF) and SiO_2 nanoparticles on fluorescence of plant gametic cells (immature microspores). The data were recorded by a BD FACSJazz® cell sorter after cell irradiation by blue laser (488 nm). A significant difference of fluorescence was observed between gametic cells after 1 hour incubation in suspension of SiO_2 nanoparticles and the control gametic cells. It was observed that fluorescence intensity of gametic cell was higher with ELF EMF treatment in comparison to control cells, but it was statistically significant only for cells treated with electromagnetic radiation field with density 100 μT ($p < 0.01$) and 400 μT ($p < 0.01$). A different effect of ELF EMF were observed in cells incubated in SiO_2 nanoparticle suspension; interaction of the factors resulted in lower cell fluorescence in comparison to control cells. The present study showed that SiO_2 nanoparticles may be a source of oxidative stress, but jointly with 50 Hz electromagnetic field they can serve as an efficient antioxidant.

Key words: *50 Hz electromagnetic radiation, immature microspores, silicon dioxide nanoparticles, flow cytometry, oxidative stress.*

INTRODUCTION

Electromagnetic fields and SiO_2 nanoparticles have been on earth for millions of years and presently they are important environmental factors, especially in urban areas. Extremely low frequency electromagnetic fields (ELF EMF) occur primarily from 50 and 60 Hz electric power lines and from electric devices and installations in buildings. Several studies have been conducted (Massot *et al*., 2000; Espinosa *et al*. 2006; Cui *et al*., 2012) on effect of oxidative stress on animal brain cells, including on the cell membrane, after exposure in ELF EMF (50 Hz). It is known that exposure to ELF EMF in a large frequency range (4–50 Hz) on human can be visualised by electroencephalograms (EEG). It is possible to alter the human EEG activity of alpha and beta bands with exposure of human to ELF EMF at corresponding frequencies, depending on the order and period of EMF conditions (Cvetkovic and Cosic, 2009). The ELF EMF influence on oxidative processes in human neutrophils has been studied a lot (Roy *et al*., 1995; Noda *et al*., 2000; Nawrocka *et al*., 2006). There are also studies showing that ELF EMF has influence on plant cell membranes, changing plant cell growth and development, and cell apoptosis, which is caused by cell oxidative stress (Piacentini *et al*., 2004, Shabrangi *et al*., 2010).

Nanoparticles are defined as particles with size less than 100 nm (Ball, 2002) and include natural, incidental and engineered nanoparticles. SiO_2 nanoparticles are the most common nano-sized particles in urban areas (Veliu and Syla, 2008; Campos-Ramos *et al*., 2011; Wang and Pui, 2011). The potential toxicity of nanoparticles has currently raised much discussion in the public and among researchers. A consistent body of evidence shows that nano-sized particles, which are taken up by a wide variety of mammalian cells types, are able to cross the cell membrane and become internalised (Novak and Bucheli, 2007). Silicon dioxide nanoparticles exert dissimilar cytotoxic effects on mammalian cells. They induce cytotoxicity by oxidative stress in human epithelial cells and in human lung cancer cells (Lin *et al*., 2006, Eom and Choi, 2011). The influence of nanoparticles on plant cells depends on type, size and concentration of particles, evaluated plant species and physiological processes in cells (Ma *et al*., 2010, Wei *et al*., 2010, Kokina *et al*., 2013, Kalteh, 2014). Only some effects of SiO_2 nanoparticles on plants are known, such as promotion

of seed germination, improved resistance to salinity stress and effect on root growth, (Hoecke *et al*., 2009, Siddiqui *et al*., 2015). The effects of SiO_2 nanoparticles on plants are being discussed.

Flow cytometry methods (FCM) are widely used for detection of changes of cell physiological state. FCM allows to evaluate a large number of cells in a very short time, and the obtained results are statistically significant and representative of the whole population (Dožel *et al*., 2007, Bargmann and Birnbaum, 2009; Galbraith, 2010). FCM on the basis of cell relative fluorescence can estimate changes in more than 20 parameters for each cell, in contrast to other methods used for determination of influence of environmental pollution — physiological, biochemical, morphological, and cytogenetic (Nabais *et al*., 1999, Yilmaz and Zengin, 2004). FSM can be used to determine effect of one or a few environmental factors, or sum of multifactorial influence on one or some traits. FCM has been successfully used for estimation of several plant cell parameters, including cell oxidative stress (Djaković and Jovanović, 2003, Dožel *et al*., 2007, Dimpka *et al*., 2012).

Studies have not been made on the combined effects of an ELF field and SiO_2 nanoparticles on oxidative stress in plant gametic cells. The aim of this study was to determine the combined effects of 50 Hz ELF EMF and SiO_2 nanoparticles on intracellular concentration of reactive oxygen species in plant gametic cells (immature microspores).

MATERIALS AND METHODS

Plant material and immature microspore cell culture preparation. Plant material. Potted donor plants of cyclamens (*Cyclamen persicum)* were grown in a greenhouse and then for a month in growing room conditions: 16 h photoperiod, 3000 lx light intensity, +20 ± 2 °C temperature, humidity ~60%. The optimal stages of microspores were determined by a light microscope with magnification $\times 10^3$ (Barnabás, 2003; Jacquard *et al*., 2003). Buds containing more than 80% microspores in the early and middle uninucleate developmental stage (5–6 mm diameter at its widest point) were collected from donor plants (Fig. 1). For microspore synchronisation buds were preserved in a growing chamber at +4 °C for two weeks in dim light, humidity ~80% (Grauda *et al*., 2014).

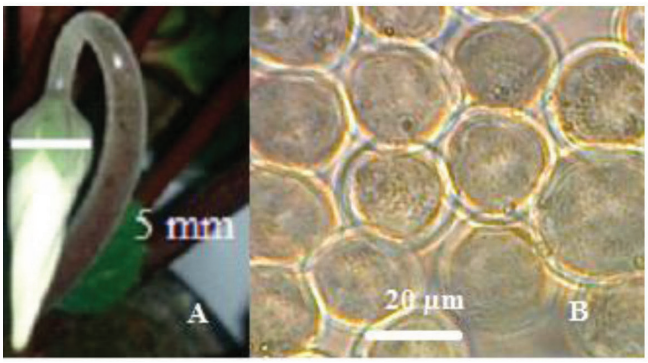

Fig. 1. Cyclamen persicum gametic cells. A, buds containing more than 80% gametic cells (microspores) in early and middle uninucleate developmental stages. B, gametic cells in the uninucleate developmental stage.

Preparation of immature microspore cell cultures was based on the modified method of Kasha *et al*. (2003). Collected buds were placed in a Waring Blender 8011 and ground in 0.3 M solution of D-mannitol in mode No. 2 up to five times for 20 seconds each till visually homogeneous suspension. The samples were filtered through mesh (50 µm) three times and then the acquired liquid was collected into 45 ml centrifuge plastic tubes and centrifuged (Eppendorf Centrifuge 5810R) for 15 min at 4 °C, 900 rpm. After centrifugation the liquid phase was decanted, and the sediment (cells) was diluted with 45 ml 0.3 M D-mannitol solution and centrifuged one more time for 15 min at 4 °C, 900 rpm. 1 ml of cell sediment contained about 600 000 cells (Kasha *et al*., 2003). The liquid phase was poured off and 1 ml of cell sediment was suspended in 4 ml liquid MS medium (Murashige and Skoog, 1962) and mixed in cultivation tubes. The cell culture quality was determined by light microscope (magnification $\times 10^3$) (Fig. 1).

A suspension of nanoparticles was prepared by dilution of silicon dioxide (SiO_2) nanoparticles (SiNPs) (Sigma –Aldrich inc., size 10–20 nm, purity 99.5%) in distilled water in proportion 1 mg per 1 ml. After dilution the suspension flask was placed in a Bandelin® RK-31 ultrasonic bath (frequency 35 kHz, effective US power 40 W) for 30 min sonification.

Experimental cultures were made by adding 1 ml of SiNPs suspension to 4 ml of cell suspension. Then cells were incubated in speed shaking regime for 1 hour. The control cells were incubated in the same conditions without adding SiNPs. The experimental cultures before flow cytometer analysis were filtered through a flow cytometry-pass filter (mesh 40 µm).

Exposure to electromagnetic radiation. A 50 Hz electromagnetic field (sinusoidal) was provided by a specific-shape signal generator and through an extremely low-frequency amplifier fed into a vertically positioned induction coil. Applied B-field monitoring was ensured throughout the whole experiment by magnetic flux density measurements in the place of the coil using a three-axis magnetometer THM1176 (Metrolab Technology, Switzerland). Samples of experimental cultures were placed into the induction coil and exposed to time-varying electromagnetic fields for 30 minutes at 20 °C. The control samples were not exposed. Density of the electromagnetic field exposure was 50, 100, 200 and 400 µT. Relative fluorescence units (RFU ± SE) of *Cyclamen persicum* gametic cells were observed for gametic cells without (GC) and after 1 hour incubation with suspension of SiO_2 nanoparticles (GC+SiO_2).

Measurement of cell relative fluorescence. A BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function was used to test relative fluorescence of plant cells. The device was equipped with an 100 µm nozzle and phosphate-buffered saline (BD Pharmingen™ PBS,

BD Biosciences, USA) was used as a sheath fluid. Cell counting events were triggered by a forward-scattered signal. The excitation of the cell fluorescence was made by a 488 nm Coherent Sapphire Solid State (blue) laser. The fluorescence emission was measured at 530 nm (bandwidth 30 nm) and 585 nm (bandwidth 29 nm). Before measurements, the flow cytometer was calibrated using Sphero^{TM} rainbow calibration particles (3.0–3.4 μm, BD Biosciences, USA) in phosphate buffered saline (PBS). The calibration was considered successful if the coefficient of variance (CV) of the calibration particle relative fluorescence did not exceed 3%. The mean fluorescence intensity in relative fluorescence units (RFU) for each cell suspension sample was recorded in the 585 channel. Cell counts were gated by the intensity in both fluorescence-detecting channels to include approximately 90% of target cells. The intensity of fluorescence was expressed in arbitrary logarithmic units. 10×10^3 gated cells were analysed from each sample. Statistical calculations were made using MS Excel software. The significance level of $p = 0.05$ was used in all analyses.

RESULTS

Density of background magnetic fields in the laboratory used for preparation of cyclamens gametic cells culture was 90 nT. As the background's EMF density was in the microtesla level, it can be considered insignificant and, probably, did not affect gametic cell response during sample preparation and testing. Data (Figs. 2 and 3, Table 1) are given as mean ± SE, n = 23 independent measurements. A significant difference of fluorescence was observed only between control gametic cells (GC) in cultivation tubes and experimental cultures of gametic cells in cultivating tubes after 1 hour incubation with suspension of SiO_2 nanoparticles (GC+SiO_2) (Table 1). Mean fluorescence intensity rate (RFU ± SE) of *Cyclamen persicum* gametic cells for GC cultures was (831.0 ± 4.2) RFU and for the GC+ SiO_2 cultures (850.8 ± 4.9) RFU ($p < 0.01$). It was observed that fluorescence intensity in cells was higher under the applied electromagnetic field than in control cells (Fig. 2). However, a statistically significant difference was found only for cells treated with an electromagnetic radiation field with density 100 μT (< 0.01) and 400 μT ($p < 0.01$). The different effect was observed for GC+SiO_2 experimental cultures. Fluorescence in the GC+SiO_2 cultures was lower than in the control (Fig. 3). A significantly lower relative fluorescence (RF) was observed in all exposed cultures in comparison to that in the control.

DISCUSSION

In this investigation cyclamen (*Cyclamen persicum*) gametic cells (immature microspores) were used as a model for two reasons: cyclamens are dicotyledonous plants that have previously (Shabrangi *et al*., 2010) been observed to be sensitive to effect of ELF-EMF, and their anthers contain a large supply of good quality young gametic cells. Plant cell fluorescence is related to the presence of fluorescent

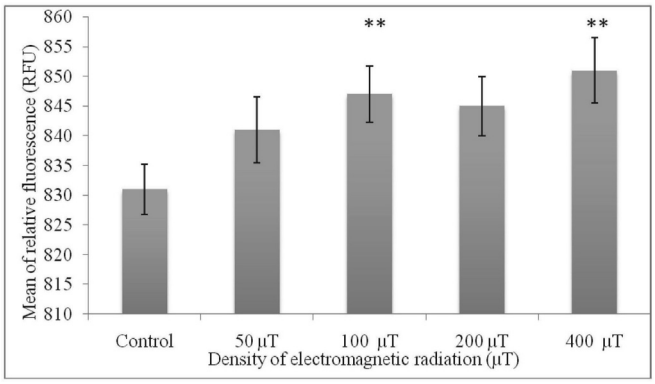

Fig. 2. Relative fluorescence (RF) of *Cyclamen persicum* gametic cells under effect of 50 Hz EMF. Control – not exposed; 50,100, 200, and 400 μT density of electromagnetic field exposure by 50 Hz MF for 30 minutes. Statistical analysis was carried out using the Student's paired t test. $^{**}p < 0.01$ versus control cultures. Data represent mean ± SE, n = 23 independent measurements.

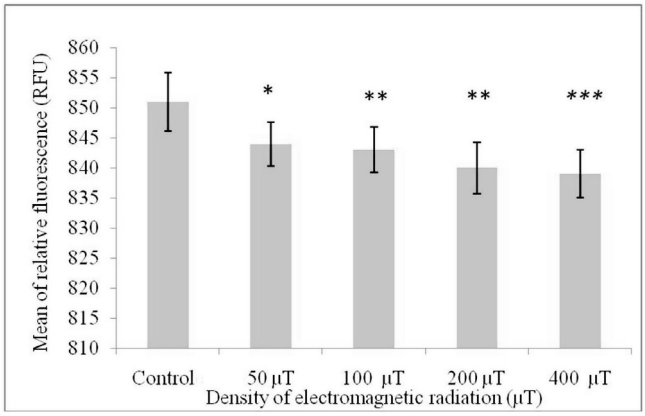

Fig. 3. Relative fluorescence (RF) of *Cyclamen persicum* gametic cells incubated in presence of SiO_2 nanoparticles after effect of 50 Hz EMF. Control – not exposed; 50, 100, 200, and 400 μT density of electromagnetic field exposure to 50 Hz MF for 30 minutes. Statistical analysis was carried out using the Student's paired t test. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 001$ versus control cultures. Data represent mean ± SE, n = 23 independent measurements.

Table 1

MEAN RELATIVE FLUORESCENCE (RFU ± SE) OF *Cyclamen persicum* GAMETIC CELLS WITH AND WITHOUT INCUBATION IN PRESENCE OF SIO_2 NANOPARTICLES AFTER EXPOSURE TO EMF

Density EMF of exposures	GC (RFU±SE)*	GC+SiO_2 (RFU±SE)**	Sig.***
Control	831.0 ± 4.2	850.8 ± 4.9	$p < 0.002$
50 μT	840.7 ± 5.5	844.5 ± 3.5	$p > 0.05$
100 μT	846.6 ± 4.6	843.4 ± 3.8	$p > 0.05$
200 μT	845.4 ± 5.0	840.2 ± 4.2	$p > 0.05$
400μT	851.1 ± 5.5	838.8 ± 4.0	$p > 0.05$

* gametic cell RFU,

** gametic cell RFU after 1 hour incubation with suspension of SiO_2 nanoparticles,

*** significance of differences between experimental cultures.

pigments in cells: proteins such as histones, cell life process products, for example, peroxidise fluorescent proteins in chloroplasts, etc. Fluorescence of cells varies depending on the plant species and physiological status of cells (Neumann and Gabel, 2002; Kimura, 2005; Bargmann and Birnbaum, 2009; You *et al*., 2015). Plant cell fluorescence changes can indicate infection processes in plants and cell apoptosis (Vigneswaran *et al*., 2009; Martinez *et al*., 2010, Carter *et al*., 2013). Young gametic cells were used for all experiments to avoid unwanted fluorescence of chloroplasts, infected and apoptotic cells. Thereby, the level of cell RF could be linked only with altered cell physiological state.

Higher gametic cell fluorescence after exposure in ELF-EMF has been observed in other studies. ELF EMF causes cell stress reactions (increase of cell fluorescence intensity). Cell exposure to ELF-EMF causes changes in oxidant and antioxidant content in cells, which induces different biological effects, such as genotoxicity (Simkó, 2007) and reduction of plant biomass (Shabrangi *et al*., 2010).

Nanotoxity of nanoparticles is related to their size. SiNPs with size 10–20 nm, as used in this study, are toxic to cells (Djaković and Jovanović; 2003, Ma *et al*, 2010; Dimkpa *et al*., 2012; Reijnders, 2012). The addition of particles of that size can cause increase of cell oxidative stress, which in turn induces increased fluorescence of cells. This was observed in the control cultures (Fig. 3), but when in addition exposed to ELF EMF, the RF was lower. It is possible that the SiNPs formed conglomerates that affect the cells as stress inhibitors. SiNPs have been observed to be an excellent treatment against salinity stress (Kalteh *et al*., 2014). It is well known that SiO_2 nanoparticles offer a versatile scaffold to develop a highly efficient antioxidant with gallic acid (Deligianakis *et al*., 2012). They also may affect changes in the cell membrane peroxidase complex and cell membrane permeability (Karim *et al*., 2012).

The present study showed that SiO_2 nanoparticles can be a source of oxidative stress, but jointly with 50 Hz electromagnetic field they can serve as an efficient antioxidant.

ACKNOWLEDGMENTS

The study was financially supported by the European Social Fund, project No. 2013/0060/1DP/1.1.1.2.0/13/APIA/VIAA/041. We wish to thank the Professor of Rīga Technical University Yuri Dekhtyar for consultations in field of SiNPs influence analysis and colleagues from the National Botanic Garden of Latvia for plant material.

REFERENCES

Ball, P. (2002). Natural strategies for the molecular engineer. *Nanotechnology*, **13**, 15–28.

Bargmann, B. O. R., Birnbaum, K. D. (2009). Positive fluorescent selection permits preside, rapid and in-depth overexpression analysis in plant protoplasts. *Plant Physiol.*, **149**, 1231–1239.

Barnabịs, B. (2003). Protocol for producing doubled haploid plants from anthers culture of wheat *Triticum aestivum* L.). In: Maluszymski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds.). *Doubled Haploid Production in Crop Plants.* Kluwer Academic Publishers, Dordrecht, pp. 65–70.)

Campos-Ramos, A., Aragon-Pina, A., Alastuey, A., Galindo-Estrada, I., Querol, X. (2011). Levels, compositions and source apportionment of rural background PM_{10} in western Mexico (State of Colima). *Atmosph. Poll. Res*., **2**, 409–417.

Carter, A. D., Bonyadi, R., Miriam, L., Gifford, (2013). The use of fluorescence-activated cell sorting in studying plant development and environmental responses. *J. Dev. Biol*., **57**, 545–552.

Cui, Y., Ge, Z., Rizak, J. D., Zhai, C., Zhou, Z., Gong, S., Che, Y. (2012). Deficits in water maze performance and oxidative stress in the hippocampus and striatum induced by extremely low frequency magnetic field exposure. *PLoS One,* **7**, e32196.

Cvetkovic, D., Cosic, I. (2009). Alterations of human electroencephalographic activity caused by multiple extremely low frequency magnetic field exposures. *Med. Biol. Eng. Comput.,* **47,** 1063–1073.

Deligiannakis, Y., Sotiriou, G. A., Pratsinis, S. E. (2012). Antioxidant and antiradical SiO_2 nanoparticles covalently funtionalized with gallic acid. *ASC Apl. Mater. Interfases*, 6609–6617.

Dimkpa, C. O., McLean, J. E., Latta, D .E., Manangó, E., Britt, D. W., Johnson, W. P., Boyanov, M. I., Anderson, A. J. (2012). CuO and ZnO nanoparticles; phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. *J. Nanopart. Res*., **14** (9), 1–15.

Djaković, T., Jovanović, Z., (2003). The role of cell wall peroxidase in the inhibition of leaf and fruit growth. *Bulg. J. Plant Physiol. Special Issue*, 264–272.

Dožel, J., Greilhuber, J., Suda, J., (2007). Flow cytometry with plants: An overview. In: Doležel, J., Greilhuber, J., Suda, J. (eds.). *Flow Cytometry With Plant Cells.* WILEY-VCH Verlag, GmbH&Co, KGaA, pp. 41–65.

Eom, H.-J., Choi, J. (2011). SiO_2 nanoparticles induced cytotoxity by oxidative stress in human bronchial epithelial cell, Beas-2b. Available at: http://dx.dol.org/10.5620/eht.2011.26.e201013.

Espinosa, J. M., Liberti, M., Lagroye, I., Veyret, B. (2006). Exposure to AC and DC magnetic fields induces changes in 5-HT1B receptor binding parameters in rat brain membranes. *Bioelectromagnetics,* **27,** 414–422.

Jacquard, C., Nolin, F., Hécart, C., Grauda, D., Rashal, I., Dhondt-Cordelier, S., Sangwan, R. S., Devaux, P., Mazeyrat-Gourbeyre, F., Clément, C. (2009). Microspore embryogenesis and programmed cell death in barley: Effects of copper on albinism in recalcitrant cultivars. *Plant Cell Rep.*, **28**, 1329–1339.

Hoecke, K. V., Quik, J. T. K., Mankiewicz-Boczek, J., Schamphelaere, K. A. C. D., Elsaesser, A., Meeren, P. V. der, Barnes, C., Howard, C. V., Meent,D. V. D., Rydzynski, K., Dawson, K. A., Salvati, A., Lesniak, A., Silversmit, G., Samber, B. D., Vincze, L., Janssen, C. R. (2009). Fate and Effects of CeO_2 Nanoparticles in aquatic ecotoxicity tests. *Environ. Sci. Technol.,* **43** (12), 4537–4546.

Galbraith, D.W. (2010). Flow cytometry and fluorescence-activated cell sorting in plants: The past, present, and future. *Biomédica*, **30**, 65–70.

Grauda, D., Miķelsone, A., Ļisina, N., Žagata, K., Ornicāns, R., Fokina, O., Lapiņa, L., Rashal, I. (2014). Anther culture effectiveness in producing doubled haploids of cereals. *Proc. Latvian Acad. Sci. Section B,* **68** (3/4), 142–147.

Kalteh, M., Zarrin, T. A., Shahram, A., Maryam, M. A., Alireza, F. N. (2014). Effect of silica nanoparticles on Basil *(Ocimum basili-cum)* under salinity stress. *J. Chem. Health Risks,* **4** (3), 49–55.

Kan, A. T., Tomson, M. B. (1999). Ground water transport of hydrophobic organic compounds in the presence of dissolved organic matter. *Environ. Toxicol. Chem*., **9**, 253–263.

Karim, Z., Adnan, R., Ansari, M. S., (2012). Low concentration of silver nanoparticles not only enhances the activity of horseradish peroxidase but alter the structure also. PLoS ONE, **7** (7); e41422.

Kasha, K. J., Simon, E., Oro, R., Shim, Y. S., (2003). Barley isolated microspore culture protocol. In: Maluszynski, M., Kasha, K. J., Forster, B.

P., Szarejko, I. (eds.). *Doubled Haploid Production in Crop Plants. A. Manual.* Kluwer Acad. Publ., Dordrecht, Boston, London, pp. 43–47.

Kimura, H., (2005). Histone dynamics in living cells revealed by photobleaching. *DNA Repair* (Amst.), **4**, 939–950.

Kokina, I., Sļedevskis, Ē., Gerbreders, V., Grauda, D., Jermaļonoka, M., Valaine, K., Gavarāne I., Pigiņka I., Filipovičs M., Rashal I. (2013). Reaction of flax (*Linum usitatissimum* L.) calli culture to supplement of mediumby carbon nanoparticles. *Proc. Latvian Acad. Sci. Section B*, **66** (415), 220–209.

Lin W., Huang Y., Zhou X.-D., Ma, Y. (2006). *In vitro* toxity of silica nanoparticles in human lang cancer cells. *Tox. Appl. Pharm.,* **217**, 252–259.

Lin, D., Xing, B., (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environ. Poll.*, **150**, 243–250.

Ma, X., Geisler-Lee, J., Deng, Y., Kolmakov, A., (2010), Interaction between engineered nananoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. *Sci. Total Environ*., **408**, 3053–3061.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.*, **15**, 473–497.

Martinez, M. M., Reif, R. D., Pappas, D. (2010). Early detection of apoptosis in living cells by fluorescence correlation spectroscopy. *Anal. Bioanal. Chem*., **396**, 1177–1185.

Nabais, C., Feritas, H., Hagemeyer, J. (1999). Dendroanalysis: A tool for biomonitoring environmental pollution. *Sci. Total Environ*., **232** (1–2), 33–37.

Nawrocka, H., Poniedzialek, B., Jaroszyk, F., Wiktorowicz, K. (2006). Effects of low intensity magnetic fields and red light on respiratory burst of neutrophils. *Pol. J. Environ. Stud*., **15** (4), 28–30.

Neumann, M., Gabel, D., (2002). Simple method for reduction of autofluorescence in fluorescence microscopy. *J. Histochem. Cytochem*., **50** (3), 437–439.

Noda, Y., Mori, A., Liburdy, R. P., Packer, L. (2000). Magnetic fields and lipoic acid influence the respiratory burst in activated rat peritoneal neutrophils. *Pathophysiology*, **7**, 137–141.

Nowack B., Bucheli T. D. (2007). Occurrence, behavior and effects of nanopaecicles in the environment. *Environ. Poll*., **150**, 5–22.

Piancentini, M. P., Piatti, E., Fraternale, D., Ricci, D., Albertini, M. C., Accorsi, A. (2004). Phospholipase C-dependent phosphoinositide breakdown induced by ELF-EMF in *Paganum harmala* calli. *Biochimie*, **86** (4–5), 343–349.

Reijnders, L. (2012). Hazards of TiO_2 and amorphous SiO_2 nanoparticles. In: *Toxic Effects of Nanomaterials.* Kahan, H. A, Arif, I. A. (eds.). Bentham Science Publishers, pp. 85–96.

Roy, S., Noda, Y., Eckert, V., Traber, M. G., Mori, A., Liburdy, R., Packer, L. (1995). The phorbol 12-myristate 13-acetate (PMA)-induced oxidative burst in rat peritoneal neutrophils is increased by a 0.1mT (60 Hz) magnetic field. *FEBS Lett*., **376**,164–166.

Siddiqui, M. H., Al-Whaibi M. H., Firoz M., Al-Khaishany, M. Y. (2015). Role of nanoparticles in plants. In: *Nanotechnology and Plant Sciences.* Siddiqui, M. H., Al-Whaibi M. H., Firoz (eds.). Springer International Publishing AG, Switzerland, pp. 19–36.

Simkó, M. (2007). Cell type specific redox status is responsible for diverse electromagnetic field effects. *Curr. Med. Chem.*, **14** (10), 1141–1152.

Shabrangi, A., Majd, A., Sheidai, M., Nabyouni, M., Dorranian D. (2010). Comparing effects of extremely low frequency electromagnetic fields on the biomass weight of C3 and C4 plants in early vegetative growth. In: *Electromagnetics Research Symposium Proceedings, Cambridge, USA, July 5–8, 2010*. Cambridge, pp. 593–598.

Van Hoecke, K., De Schamphelaere, K. A. C., Vander Meeren, P., Licas, S., Janssen, C. R., (2008). Ecotoxicity of silica nanoparticles to the green alga *Pseudokirchneriella subcapitata*: Importance of surface area. *Environ. Toxicol. Chem*., **27**, 410–420.

Vigneswaran, N., Koh, S., Gillenwater, A., (2009). Incidental detection of an occult oral malignancy with autofluorescence imaging: A case report. *Head Neck Oncol*., **1** (37), available at: www.headandneckoncology.org/conten/1/1/37.

Veliu, A., Syla, A., (2008). Air pollution with particulate matter and heavy metals of Kosova thermal power plant. *J. Int. Environ. Appl. Sci.*, **3** (4), 280–287.

Wang, J., Pui, D. Y. H., (2011). Characterization, exposure measurement and control for nanoscale particles in workplaces and on the road. *J. Phys. Conference Series*, **304** (012008), 1–14.

Wei, C., Zhang, Y., Guo, J., Han, B., Yang, X., Yuan, J. (2010). Effects of silica nanoparticles on growth and photosynthetic pigment contents of Scenedesmus obliquus. *J. Environ. Sci*. (China), **22** (1), 155–60.

Yilmaz, S., Zengin, M. (2004). Monitoring environmental pollution in Erzurum by chemical analysis of Scott pine (*Pinus sylvestris* L.) needles. *Environ. Int*., **29**, 1041–1047.

You, M. K., Lim, S.-H., Kim, M.-J., Jeong, Y.S., Lee, M.-G., Ha, S.-H., (2015). Improvement of the fluorescence intensity during a flow cytometric analysis for rice protoplasts by localization of a green fluorescent protein into chloroplasts. *Int. J. Mol. Sci*., **16**, 788–804.

Received 18 May 2015

ZEMFREKVENCES (50 HZ) ELEKROMAGNĒTISKĀ LAUKA UN SIO_2 NANODAĻIŅU KOMBINĒTĀ IETEKME UZ AUGU GAMETISKO ŠŪNU OKSIDATĪVO STRESU

Urbānajā vidē dzīvie organismi atrodas nepārtrauktā saskarē ar dažāda veida piesārņojumu. Gan zemfrekvences elektromagnētiskais lauks (ZF EML), gan SiO_2 nanodaļiņas ir vispārzināmi urbānās vides piesārņojuma veidi. Kombinēta zemferekvences elektromagnētiskā lauka un SiO_2 ietekme uz šūnām ir maz pētīta. Šī pētījuma mērķis bija noteikt ZF EML un bioloģiski aktīvu (diametrā mazākas par 20 nm) SiO_2 nanodaļiņu ietekmi uz augu gametiskajām šūnām. Kā modeļa objekts pētījumā tika izmantotas *Cyclamen persicum* gametiskās šūnas vienkodola attīstības stadijā. Gametiskās šūnas tika kultivētas MS barotnē. Pirms ievietošanas ZF EML šūnas 1 h tika inkubētas barotnē, kam pievienoja SiO_2 nanodaļiņu suspensiju (1 mg/10 ml). Šūnas tika apstrādātas ZF EML 50 Hz ar blīvumu 50, 100, 200 un 400 μT. Šūnu relatīvās fluorescences izmaiņas tika noteiktas ar plūsmas citometru (BD FACSJazz®). Parādīts, ka gan SiO_2 klātūtne kultivācijas barotnē gan šūnu ievietošana ZF EML statistiski ticami palielina gametisko šūnu fluorescenci. Šūnu apstrāde ar ZF EML, pēc to inkubācijas SiO_2 nanodaļiņu suspensijā, statitiski ticami samazināja šūnu relatīvo fluorescenci. Rakstā tiek apspriesti iespējamie šāda efekta iemesli.