Progress in Nutrition 2017; Vol. 19, Supplement 1: 90-97 DOI: 10.23751/pn.v19i1-S.5280

ORIGINAL ARTICLE

Influence of extremely low frequency electromagnetic field on antioxidant system and change of volatile composites in *Pinus sylvestris* L.

Nevzat Esim[1], *Ömer Kiliç*[1], *Deniz Tiryaki*[2], *Aydın Şükrü Bengü*[3]

Vocational Training High School, Bingöl University, 12000 Bingöl, Turkey - E-mail: omerkilic77@gmail.com; [2] Atatürk University, Science Faculty, 25240, Erzurum, Turkey; [3] Health Science Vocational College, Bingöl University, 12000, Bingol, Turkey

Summary. The present study investigated the effect of extremely low frequency electromagnetic fields (ELF - EMFs) from high voltage power line on the antioxidant balance and inducible volatile emissions of *Pinus sylvestris* L. needles. The samples were collected from pines just below power line (P0, 50.66 mG) and 10 meter away from same power line. They were then categorized as P0 (just below power line) and 10 meter away from the same power line (P10, 6.30 mG). ELF - EMFs inhibited superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), but increased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in needles of P0 plants. Therefore, volatile components of needles were investigated by GC-MS. The total rate of the volatile components for P0 and P10 plants was determined as 93.39% and 95.11%, respectively. α-pinene (20.74%), cyclohexene (9.20%), caryophyllene (9.10%) and bornylacetate (8.13%) were identified as main components in needles of P0 plants, and α-pinene (17.40%), bornylacetate (17.33%), cyclohexene (14.98%) and β-pinene (13.24%) in needles of P10 plants. Consequently, our findings suggest that Pine trees are sensitive to ELF - EMFs emitted from power line due to cause oxidative damage and alter the rate of volatile components in pine.

Key words: pine, reactive oxygen species, essential oil, power line, HS-SPME-GC-MS

Introduction

All live in the world in world have expressed concern that exposure to Electromagnetic fields (EMF) from mobile phone base stations and high voltage power lines may have adverse effects on their health. The biological or biochemical consequences of electromagnetic fields are entirely dependent on the frequency range and the intensity of the applied field. Extremely low frequency fields exist wherever a time-varying voltage, for example mains electricity at 50 Hz, is present, regardless of whether or not any current is flowing. The extremely low frequency field's sources are both external, such as power lines and transformer stations, and internal, such as the home electrical system, appliances and electrical equipment connected to the electrical network (1-3).

Plant productivity is minimized by the number of factors such as soil salinity, droughts, and soil erosion and widespread of disease. Electromagnetic field resulting from extremely low frequency fields (ELF - EMFs) is one of this adverse situation in the earth. Whole organisms including plants are interacted with magnetic field in day to day life. Generally, the earth acts as a magnet with their south and north poles and the natural effects of magnetic field have been changing the plant growth and yield in the globe. However, very limited studies have been conducted in biology to describe the role of ELF – EMFs (4). One possible explanation for the adverse effects of ELF - EMFs on living organisms is oxidative stress by an increase in the production of reactive oxygen species (ROS) (5,6). Oxidative stress affects the membrane structure and cell growth and can even cause cell death (7). This in turn triggers a cascade of reactions leading to cellular death. A multitude of antioxidants are regarded to be components of induced defense (8). These antioxidants include enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), which have been reported to control the oxidative stress caused by abiotic stress (9). Oxidative stress might also trigger other inducible defenses, such as the emission of volatile organic compounds including monoterpenes and sesquiterpenes (10).

Turkey, because of its geographical position at the crossing region of temperate continental and Mediterranean climates, is rich in plant diversity and coniferous that grow in different regions of the country, occupying about half of the county's total forest area (11). There are five pine species in Turkey, which are *P. brutia* Ten., *P. nigra* Arn., *P. sylvestris* L., *P. pinea* L. and *P.halepensis* Mill. (12). Pine oils are commonly used as fragrances in cosmetics industry, as flavoring additives for food and beverages industry and as scenting agents in a variety of household products (13). With growing interest in the use of essential oils in both the food and pharmaceutical industries, the systematic examination of plant extracts for these characteristics has become more and more important (14). Cones of some coniferous taxa are used in industry (15). Former studies on *Pinus* taxa were performed on the diterpenoids, triterpenoids, flavonoids and lignans (16). Studies with conifers mostly deal with the reasons for the chemical variation of pine oils from the seasonal, geographical, genotypic, and environmental points of view (17,18). Pine needles are rich in terms of vitamin C, tannins, alkaloids, essential and volatile oils. Furthermore, coniferous are very susceptible to air pollution, and the analysis of secondary metabolites allow to evaluate both the physiological state of a plant and the environmental conditions under which it is growing (19). The aim of the present study was to investigate effect of ELF - EMFs resulting from power line on the oxidative system and chemical composition of two pine trees, which grown at different distances from power line.

Material and Methods

Plant source and experimental site

The forested area used for the experiments was located in an urban environment, (38° 53' 56.17", 40° 28' 34.67). *Pinus sylvestris* samples as plant source were collected from South west of Bingöl University Campus, Bingöl/Turkey, alongside the 154 kV and 50 Hz transmission line, on 12.04.2013, at an altitude of 1150-1200 m., by O. Kilic and N. Esim. (Leg. No: 4834) (Fig. 1). The needles of the same age were taken from same parts of the two different pine trees and then used as plant source (Fig. 2). Plant material was

Figure 1. High voltage power line: an important source of electromagnetic field results from extremely low frequency field (ELF - EMFs)

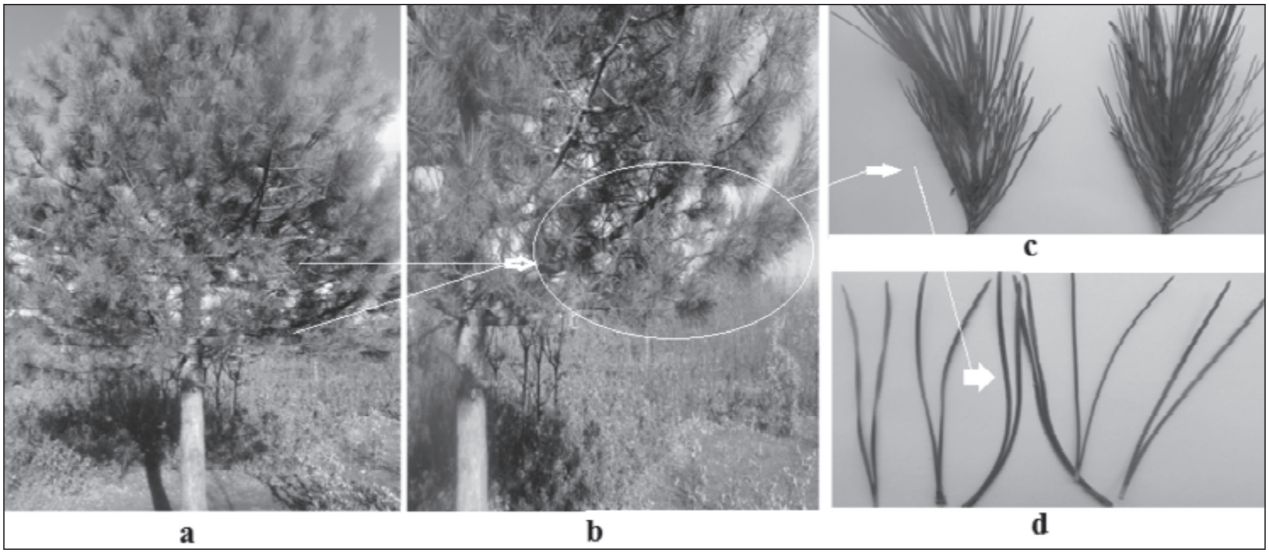

Figure 2. The needles taken from same parts of the two different pine tree. a) general view; b) sample received parts; c) sampled branchs; d) needles of samples

identified with Flora of Turkey and East Aegean Islands, volume 1 (12). The experiment was designed as a comparison of two different magnetic field strengths resulting from power line (Table 1). The power lines are approximately 15 m height and compose of five wires. Plant materials were collected just below (0 m, categorized as P0) and the 10 m away (categorized as P10) from same line. Electromagnetic fields of these areas were measured as 50.66 mG and 6.30 mG, respectively (Table 1). Some factors such as soil analysis, plant nutrient levels and soil pH were the same for both pines (data not shown).

HS-SPME procedure

Five grams powder of two plant needles (near and far to power line) were obtained by a (HS-SPME) head space solid phase micro extraction method using a divinyl benzene / carboxen / polydimethyl siloxane (DVB/CAR/PDMS) fiber, with 50/30 lm film thickness; before the analysis the fiber was pre conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of needles, previously homogenized, were weighed into a 40 ml vial; the vial was equipped with a "mininert" valve. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibratefor 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analyses thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al., 2004 (20).

GC-MS analysis

A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer

Table 1. Levels of electromagnetic fields of areas in different distances from the 154 kV high voltage transmission

	Different distance from high voltage power line (m)	
	0 m (P0)	10 m (P10)
Electromagnetic field values	50.66 mG, 50 Hz	6.30 mG, 50 Hz

P0: just below high voltage power line, P10: 10 m away from high voltage power line, mG: miligauss

(Varian Spa, Milan, Italy) was used with injector temperature, 260°C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 lm film thickness (Chrompack Italys.r.l., Milan, Italy). The oven temperature was programmed as follows: 45°C held for 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C at 2°C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisit ion range, 40 to 200 m/z; scan rate, 1 us-1. The compounds were identified using the NIST (National Institute of Standards and Technology) library (NIST/WILEY/EPA/NIH), mass spectral library and verified by the retention indices which were calculated as described by Van den Dool & Kratz 1963 (21).

Determinations of malondialdehyde, hydrogen peroxide and superoxide anion contents

Malondialdehyde (MDA) content was determined according to the method of Heath & Packer (1968) (22). Needles were weighed and homogenates containing 10 % trichloroacetic acid (TCA) and 0.65 % 2-thiobarbituric acid (TBA) were heated at 95°C for 60 minutes and then cooled to room temperature and centrifuged at 10.000 x *g* for 10 minutes. The absorbance of the supernatant was read at 532 and 600 nm against a reagent blank. MDA content was expressed as nmol g^{-1} FW. H_2O_2 were measured by monitoring the absorbance at 410 nm of the titanium-peroxide complex according to He *et al.*, (2005) (23). One gram of leaf was extracted using 10 mL cold acetone and centrifuged at 3.000 x *g* for 20 minutes. An aliquot (1 mL) of supernatant was added to 0.1 mL 20 % titanium reagent and 0.2 mL of 17 M ammonia solutions. The solution was centrifuged at 3.000 x *g* at 4°C for 10 minutes and the supernatant was discarded. The pellet was dissolved in 3 mL of 1 M sulphuric acid. The absorbance of the solution was measured at 410 nm. Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2. Superoxide anion ($O_2^{\cdot -}$) content was measured as described by Elstner & Heupel, 1976 (24). A 0.5 g aliquot of needles was ground and extracted in 2 mL of 65 mM phosphate buffer (pH 7.8).The homogenate was centrifuged at 5,000 g for 10 min at 4°C. A 1 mL aliquot of the supernatant was mixed with 0.9 mL of 65 mM phosphate buffer (pH 7.8) and 0.1 mL of 10 mM hydroxylamine hydrochloride, and then the mixture was incubated at 25°C for 20 min. Then, 1 mL of the mixture, 1 mL of 17 mM anhydrous p-aminobenzene sulfonic acid, and 1 mL of 17 mM 1-naphthylamine were mixed and then incubated at 25°C for 20 min. The absorbance was monitored at 530 nm after 3 mL of n-butyl alcohol was added to the mixture.

Antioxidant enzyme extraction and assays

Needles (0.5 g) were homogenised in 50 mM sodium phosphate buffer (pH 7.0) containing 1 % polyvinyl pyrolidine. The homogenate was centrifuged at 20,000 x *g* for 15 minutes at 4 °C, and the supernatant was to determine the activities of superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6) and peroxidase (POX) (EC 1.11.1.7). Activity of SOD was estimated by recording the decrease in optical density of nitro blue tetrazolium (NBT) dye by the enzyme (Dhindsa et al. 1981). The reaction mixture of 3 mL contained 2 mM riboflavin, 13 mM methionine, 75 mM NBT, 0.1 mM ethylene diamine tetra acetic acid (EDTA), 50 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate and 0.05 mL enzyme fraction. The reaction was started by adding riboflavin solution and placing the tubes under two 30 W fluorescent lamps for 15 minutes. A complete reaction mixture without enzyme, which gave the maximal colour, served as a control. The reaction was stopped by switching off the light and putting the tubes in the dark. A non-irradiated complete reaction mixture served as a blank. The absorbance was recorded at 560 nm, and 1 unit of enzyme activity was taken as the amount of enzyme that reduced the absorbance reading to 50% in comparison with the tubes with no enzyme (25). Activity of CAT was measured by monitoring the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2. One unit of CAT activity was defined as the amount of enzyme that used 1 mmol H_2O_2 min^{-1} (26). Activity of POX was measured by monitoring the increase in the absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H_2O_2. One unit of POX activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 min^{-1} (27).

Statistical analysis

All experiments were performed three times with the same samples. Data were analyzed by analysis of variance (ANOVA), and means were compared by Duncan's multiple range test at P ≤ 0.05.

Results and Discussion

The result of study was showed that electromagnetic fields resulting from extremely low frequency electromagnetic field (ELF-EMFs) were able to trigger excessive generation of superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) in the needles (Table 2). ELF - EMFs increased the $O_2^{\cdot -}$ and H_2O_2 contents by 17 and 23 % in needles of P0 (just below power line; 50.66 mG) and P10 (meter away from power line; 6.36 mG) plants, respectively (Table 2). ELF - EMFs significant increased (83 %) in lipid peroxidation (MDA content) of P0 plants compared with P10 plants (Table 2). Also, the results of enzyme activities showed that SOD, CAT, and POX were inhibited in P0 and compared to P10 plants (Table 3). Furthermore, some importantly volatile components were modified in needles of P0 and P10 plants due to ELF – EMFs (Table 4).

Reactive oxygen species, such as $O_2^{\cdot -}$ and H_2O_2, are regularly generated by various oxygen metabolisms primarily in chloroplasts, mitochondria and peroxisomes (28). In normal conditions, accumulation of ROS can be effectively controlled by the antioxidant enzymes in these organelles (29). However, the risk of serious cellular damage may arise when the ROS is overproduced under stress conditions. $O_2^{\cdot -}$ and H_2O_2 affect stress responses in two different ways. One way is that they can influence expression of a number of genes and, in this case, they act as a signal molecule. Another way is that, they affect many cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (30). In the normal conditions, in order to keep ROS under control, plants display a balance between ROS and antioxidant enzymes such as SOD, APX, and CAT. Within a cell, SOD constitutes the first line of defense against $O_2^{\cdot -}$ by rapidly converting $O_2^{\cdot -}$ to O_2 and H_2O_2, which are further oxidized to molecular oxygen and H_2O by the enzymes of CAT and POX[9]. Although there are many studies about possible roles of ELF - EMFs on oxidative damage in animals and humans (31,32), there is no information about the role of ELF - EMFs on oxidative damage in plants. The role of ELF - EMFs on oxidative damage in plants was investigated for the first time in this study. Results of the present study are similar with those of previous studies, which were performed on animals and humans (31). Namely, ELF – EMFs induced oxidative stress by increasing ROS

Table 2. Effects of distinct electromagnetic fields on oxidative stress parameters of pine needles

Groups	Electromagnetic fields value	$O_2^{\cdot -}$ content (ng. g^{-1})	H_2O_2 content (ng. g^{-1})	MDA content (nmol. ml^{-1})
P10	6.30 mG	1.72 ± 0.01 b	41 ± 0.3 b	1,16 ± 0.02 b
P0	50.66 mG	1,84 ± 0.02 a	46 ± 0.5 a	2,11 ± 0.04 a

P0: just below high voltage power line, P10: 10 m away from high voltage power line, mG: miligauss.
Means within each plant group followed by the same letter are not significantly different (P<0.05) according to Duncan's multiple range test ± represents standard error (SE) of the mean (n = 3)

Table 3. Effects of distinct electromagnetic fields on antioxidant enzymes activity of pine needles

Groups	Electromagnetic fields value	SOD activity (U.g^{-1})	CAT activity (U.g^{-1})	POX activity (U.g^{-1})
P10	6.30 mG	813 ± 11 b	90 ± 9 b	100 ± 12 b
P0	50.66 mG	891 ± 15 a	260 ± 15 a	400 ± 40 a

P0: just below high voltage power line, P10: 10 m away from high voltage power line, mG: miligauss.
Means within each plant group followed by the same letter are not significantly different (P<0.05) according to Duncan's multiple range test ± represents standard error (SE) of the mean (n = 3)

generation and by decreasing antioxidant enzymes. This showed that ELF – EMFs is harm for plants.

In this study, α-pinene (20.74%), cyclohexene (9.20%), caryophyllene (9.10%), bornylacetate (8.13%) were identified as main components in P0 plants. Conversely, main components of P10 plants were identified as α-pinene (17.40%), bornylacetate (17.33%), cyclohexene (14.98%) and β-pinene (13.24%) (Table 4). ELF - EMFs inhibited bornylacetate, cyclohexene and β-pinene, but increased α-pinene and caryophyllene. This showed that especially main components such as α-pinene and caryophyllene may be effective in obtaining of tolerance against to stress. Tumen et al. 2010 demonstrated that α-pinene (14.76%, 45.36%, 47.09%) was as the main constituent of *P. slyvestris, P. nigra, P. halepensis*, respectively. Similarly, α-pinene (12.96%, 33.29%, 32.96%, 25.56%, 9.00%) was determined to be main component of *P. resinosa*, *P. flexilis*, *P. strobes*, *P. parviflora* and *P. mugo* subsp. *mugo* (32). In *Pinus petula* caryophyllene oxide (14.8%), β-phellandrene (12.1%); in *P. ponderosa* β-pinene (38.2%), α-pinene (13.0%); in *P. pumila* α-pinene (18.3%), δ-3-carene (10.4%); in *P. rigida* β-pinene (15.2%), α-pinene (11.1%); in *P. rudis* β-pinene (21.4%), and caryophyllene oxide (20.0%) were determined as main constituents (14). Therefore, *Pinus* taxa can be separated into two groups; first group contains a large amount of α-pinene, and the second contains little α-pinene.

In this study, high amounts of monoterpenes (54.94% - 66.86%) and sesquiterpenes (14.80% - 6.44%) were detected in needles of the P0 and P10 plants, respectively (Table 4). Monoterpenes such as α-pinene, limonene, *p*-cymene increased in needles of P0 plants by ELF - EMFs but β-pinene and bornyl acetate was decreased (Table 4). In addition, sesquiterpenes such as α-cubebene and caryophyllene increased in needles of P0 plants by ELF - EMFs (Table 4). The terpenes constitute the largest class of secondary products. Terpenes have a well-characterized function in plant growth or development and so can be considered primary rather than secondary metabolites. Terpenes are toxins and feeding preventions to many plant feeding insects and mammals; thus they appear to play important defensive roles in the plant kingdom (33). The results of the present study showed that plants tend to increase the content of terpenes such as monoterpens

Table 4. The effect of electromagnetic fields on chemical composition of *P. sylvestris* samples

Monoterpenes	**RRI**	***P. sylvestris* (P0) %**	***P. sylvestris* (P10) %**
α-pinene	1023	**20.74**	**17.40**
β-pinene	1065	0.55	3.69
β-myrcene	1090	1.11	0.53
Limonene	1108	2.74	-
p-cymene	1112	2.22	-
3-carene	1120	0.78	4.81
β-phellandrene	1125	1.34	-
α-terpinolene	1128	1.36	2.52
Bornyl acetate	1132	**8.13**	**17.33**
Limonene	1108	2.74	-
β-pinene	1270	5.01	**13.24**
β-phellandrene	1276	2.82	1.55
Camphene	1284	-	0.50
Sabinene	1338	-	1.00
γ-terpinene	1346	1.41	0.43
β-ocimene	1368	0.49	0.95
Santolinatriene	1384	1.27	2.61
Terpinolene	1396	0.11	0.13
1,8-cineole	1407	-	0.03
α-terpinene	1573	0.12	0.10
Naphthalene	1871	-	0.01
Isobornylacetate	1884	-	0.03
	Total	**54.94**	**66.86**
Sesquiterpenes	**RRI**	***P. sylvestris* (P0) %**	***P. sylvestris* (P10) %**
α-cubebene	1309	1.87	-
Copaene	1314	-	2.09
α-bourbonene	1330	0.61	-
Spathulenol	1555	-	0.23
α-cubebene	1589	0.13	0.05
Caryophyllene	1841	**9.10**	3.17
β-farnesene	1863	0.10	0.10
γ-cadinene	1879	0.26	0.02
α-muurolene	1896	0.17	0.08
α-humulene	1908	0.65	-
δ-cadinene	1918	0.93	0.15
Germacrene D	1935	0.75	0.55
Bicyclogermacrene	**1962**	**0.23**	-
	Total	**14.80**	**6.44**

Continued

Others	**RRI**	***P. sylvestris* (P0) %**	***P. sylvestris* (P10) %**
Benzene, 1-methyl-4	1095	1.33	-
1,3,5-cycloheptatriene	1102	3.13	0.12
Mentha-2,4 (8)-diene	1170	0.88	-
Cyclohexene	1175	**9.20**	**14.98**
1,3,6-octatriene	1182	-	2.98
Bicyclo (4.1.0) hept-3-ene	1235	5.88	0.58
Bicyclo (3.1.0) hexane	1244	1.24	-
1,6-octadiene	1259	1.45	-
Undecanal	1296	-	2.09
Tricyclene	1357	0.22	-
Bicyclo (4.4.0) dec-1ene	1374	0.89	-
α-guaiene	1793	0.50	-
Isoledene	1832	0.04	-
Benzaldehyde	1852	0.16	0.07
Heneicosane	2115	-	0.04
Linoleic acid	2136	0.71	-
Tricosane	2304	0.02	0.03
Hexacosane		-	0.92
	Total	**28.65**	**21.81**
	General Total	**93.39**	**95.11**

and sesquiterpens to avoid from toxic effects of high magnetic fields.

Volatile terpenes are not only defenses in their own right, but also provide a way for plants to call for defensive help from other organisms. However, there is no research in literature about the role of ELF - EMFs on alteration of volatile components in plants. The present study experiments exhibited that contents of monoterpenes and sesquiterpenes increased in *P. sylvestris* needles, which exposed to ELF - EMFs. Therefore, we contemplate that these components may have a possible positive role in obtaining of tolerance against stress.

In conclusion, the results of the present study indicated that magnetic field results from extremely low frequency field (ELF – EMF) induced-oxidative damage and modified volatile components in pine needles. Therefore, to protect the *Pinus* against adversities result from extremely low frequency fields should be taken some precaution. Some of this precaution may include the following. (I) High-voltage power lines can be taken underground (II) or the height of the poles should be further raised. However, further research is required at molecular level to gain deeper insight understanding the effects occur in plants under high- voltage power lines.

References

1. World Health Organization. World Health Organization. Environmental Health Criteria (EHC) Document on ELF Fields, Doc No. 238, downloadable from the WHO EMF Project website, 2007. www.who.int/emf.
2. World Health Organization. Environmental Health Criteria 69: Magnetic fields. 1987. Geneva.
3. Committee on the Effects of Electromagnetic Fields on Biologic Systems, http://www.environ.ie/en/Publications/Environment/EnvironmentalRadiation/FileDownLoad,2477,en.pdf.
4. Radhakrishnan R, Kumari BDR. Pulsed magnetic field: A contemporary approach offers to enhance plant growth and yield of soybean. Plant Physiol. Biochem 2012; 51:139-144.
5. Falone S, Mirabilio A, Carbone MC, Zimmitt V, Di Loreto S, Mariggio MA, Mancinelli R, Di Ilio C, Amicarelli F. Chronic exposure to 50 Hz magnetic fields causes a significant weakening of antioxidant defence systems in aged rat brain. Int. J. Biochem. Cell Biology 2008; 40: 2762-2770.
6. Hajnorouzia A, Vaezzadeha M, Ghanati F, Jamnezhada H, Nahidianb B. Growth promotion and a decrease of oxidative stress in maize seedlings by a combination of geomagnetic and weak electromagnetic fields. J. Plant Physiol 2011; 168: 1123-1128.
7. Martinez-Samano J, Torres-Durán PV, Juárez-Oropeza MA, Elinas-Vinas D, Verdugo-Diaz L. Effects of acute electromagnetic field exposure and movement restraint on antioxidant system in liver, heart, kidney and plasma of Wistar rats: a preliminary report. Int. J. Radiat. Biol 2010; 86: 1088–1094.
8. Foyer CH, Noctor, G.Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 2005; 28: 1056-1071.
9. Esim N, Atici O. Nitric oxide improves chilling tolerance of maize by affecting apoplastic antioxidative enzymes in leaves. Plant Growth Regul 2014; 72: 29-38.
10. Menendez AI, Romero, AM, Folcia AM, Martínez-Ghersa MA. Getting the interactions right: will higher ozone levels interfere with induced defenses to aphid feeding. Basic and Appl.Ecol 2009; 10: 255-264.
11. Hafizoǧlu H, Usta M. Chemical composition of coniferous wood species occurring in Turkey. Holz als Roh- und Werkstoff 2005; 63: 83-85.
12. Davis PH. Flora of Turkey and The East Aegean Islands. Edinburgh University Press, Edinburgh. 1965; 1: 465-585.

13. Sezik E, Osman U, Demirci B, Baser, KHC. Composition of the essential oils of Pinus nigra Arnold from Turkey. Turk. J. Chem 2010; 34 313-325.
14. Kurose K, Okamura D, Yataga M. Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. Flavour Fragr J. 2007; 22: 10-20.
15. Villagomez HZ, Peterson DM, Herrin L, Young, RA. Antioxidant activity of different components of pine species. Holzforschung 2005; 59: 156-162.
16. Tumen I, Hafizoglu H, Kilic A, Dönmez IE, Sivrikaya H, Reunanen, M. Yields and Constituents of Essential Oil from Cones of Pinaceae spp. Natively Grown in Turkey. Molecul 2010; 15: 5797-5806.
17. Kupcinskiene E, Stikliene A, Judzentiene A. The essential oil qualitative and quantitative composition in the needles of Pinus sylvestris L. growing along industrial transects. Environ. Pollut 2008;155: 481-491.
18. Lazutka JR, Mierauskien J, Slapšyt G, Dedonyt, V. Genotoxicity of dill (Anethum graveolens L.), peppermint (Mentha piperita L.), and pine (Pinus sylvestris L.) essential oil in human lymphocytes and Drosophila melanogaster. Food Chemistry Toxicol 2001; 39: 485-492.
19. Asta J, Jurgita S, Aida S, Eugenija K. Characteristics of essential oil composition in the needles of young Scots pine (Pinus sylvestris L.) stands growing along an aerial ammonia gradient. Chemija 2006; 17: 67-73.
20. Verzera A, Zino M, Condurso C, Romeo V, Zappala M. Solid-phase microextraction and gas chromatography/mass spectrometry for the rapid characterisation of semi-hard cheeses. Anal. Bioanal. Chem 2004; 380: 930-936.
21. Van Den Dool H, Kratz, PD. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J. Chromatog 1963; 11: 463-471.
22. Heath RL, Packer, L. Photo peroxidation in isolated chloroplast I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys 1968; 25:189-198.
23. He YL, Liu., YL, Cao, WX, Huai., MF, Xu., Huang, BG. Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. Crop. Sci 2005; 45: 988-995.
24. Elstner EF, Heupel, A. Formation of hydrogen peroxide by isolated cell walls from horseradish. Planta 1976; 130:175-180.
25. Sairam RK, Srivastava GC. Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes. Bio Plantarum 2000; 43: 381–386.
26. Green MA, Waldbusser GG, Rely SL, Emerson K, O'Donnella S. Death by dissolution: Sediment saturation state as a mortality factor forjuvenile bivalves. Limnol Oceanogr 1999; 54: 1037-1047.
27. Yee Y, Tam NFY, Wong YS, Lu, CY. Growth and physiological responses of two mangrove species (Bruguira gymnorrhiza and Kandelia candel) to waterlogging. Environ. Exp. Bot 2002; 49: 209-221.
28. Zhou ZS, Huang SQ, Guo K, Mehta SK, Zhang PC, Yang ZM. Metabolic adaptations to mercury-induced oxidative stress in roots of Medicago sativa L. J. Inorg. Biochem 2007, 101: 1-9.
29. Del Rio L, Sandalio LM, Altomare DA, Zilinskas, BA. Mitochondrial and peroxisomal manganese superoxide dismutase: differential expression during leaf senescence. J. Exp. Bot 2003; 54: 923-933.
30. Mittler, R. Oxidative stress, antioxidants and stress tolerance.Trends Plant Sci2002;7:405-410.
31. Gershenzon J, Croteau R. Terpenoids. In Herbivores: Their Interactions with Secondary Plant Metabolites, Vol. 1: The Chemical Participants, 2nd ed., G. A. Rosenthal and M. R. Berenbaum, eds., Academic Press, San Diego, CA, pp. 1992, 165-219.
32. Kilic Ö, Kocak A. Essential Oil Composition of Six Pinus L. Taxa (Pinaceae) From Canada and Their chemotaxomony. J. of Agric. Sci. and Tech. 2013; B: 67-73.
33. Taiz L, Zeiger E. Plant Physiology, Sinauer Associates, Sunderland, MA. 2002; 690 pps.

Correspondence:
Ömer Kiliç
Vocational Training High School, Bingöl University, Bingöl, Turkey, 12000
E-mail: omerkilic77@gmail.com