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学位論文題目	NOVEL APPROACHES TO DETERMINE EFFECTS OF RADIOFREQUENCY ELECTROMAGNETIC RADIATIONS ON PHYSIOLOGY AND MORPHOLOGY OF PLANTS (植物の生理及び形態に及ぼされる高周波数の電磁波の影響の解明)
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論文の要約

The wireless communication technologies are becoming popular and spreading all over the world. Mobile phones services are the most common and wireless broadband services are becoming popular at fast rates. The radiofrequency electromagnetic radiations (EMR) emitted by those services concentrates in the environment day by day and with the advancement of the technologies, network operators prefer more bandwidths and frequency spectrums. Owing to this reason, environmental existence of higher frequency EMR is increasing (i.e. Frequency range between 2 – 5.5 GHz). At present, there is a wide concern regarding EMR exposure influence on humans and other animals; however, investigations on plants are lacking.

Two distinct aquatic plant species (*Lemna minor* and *Myriophyllum aquaticum*) were investigated in order to determine the existence of effects by EMR exposure. *Lemna minor* (Duckweed) a floating type aquatic macrophyte, which is widely adopted as an environmental indicator was used for the experiment to determine EMR influence on chlorophyll fluorescence (ChF) parameters. Duckweed fronds are floating on water surface exposing whole upper leaf surface upward, forming a layer covering the water surface, facilitating uniform EMR exposure and the accurate determination of ChF parameters. Since, ChF parameters can change with less than a 10 min exposure to stress conditions; it is the best method to determine the effect of EMR on duckweeds photosystem. *Myriophyllum aquaticum* (Parrot Feather) is an emergent type perennial aquatic macrophyte, which contains soft-woody stem and vascular bundles passing through the center of the stem. The soft-woody stem of the plant facilitates convenient insertion of glass microelectrodes to the vascular bundles for electric potential (EP) studies and the preparation of plant extracts for quantification of chemical compounds. Further, the rapid growth rate (1.8 ± 0.2 cm/day) of the plant is convenient for growth studies.

The electric potentials of plants are important for rapid and long-distance communication within a plant. Changes in the electric potential (EP) within plants have been observed during the propagation of electric signals. Therefore, any factor changing the EP in a plant will disturb the signaling system of the plant. To investigate the EP properties, two 50 μm Ag/AgCl glass microelectrodes connected to an mV meter was used. Electrodes were inserted to the plants at certain distance in to the vascular tissues and EP was recorded after keeping the plant to stabilize with the electrodes.

The existence of various frequency EMR in the environment (i.e. Wifi, mobile and broadband internet access) is interfering with the experiments, since we studied a specific frequency at a time. Therefore, all the experiments were conducted inside specially designed two identical EMR-shielded anechoic chambers. Chambers are reducing plant exposure to outside EMR by over 95%. One of the chambers were used as an EMR treatment chamber and the other one for EMR prevented control experiments (sham exposure experiments). The EMR exposure was done by transmitting the EMR of a particular frequency inside EMR exposure chamber via microstrip antennas, prepared for each frequency tested. The EMR generation was done using RF signal generator and it was amplified by a linear RF amplifier. The power density received by the plants was measured using, a microstrip antenna with same EMR frequency which plants are exposed connected to a power meter via a power sensor.

Duckweed fronds were tested with 2, 2.5, 3.5, 5.5 and 8 GHz EMR frequencies and fronds were exposed to EMR for 30 min, 1 h and 24 h durations with EMR electric field strength of 45 - 50 V/m for each frequency. The ChF parameters were quantified using ChF imaging technique after dark adaptation while the EMR exposure continues. The results indicated that exposure to EMR causes a change in the non-photochemical quenching of the duckweeds. The changes varied with the frequency of the EMR and were time-varying within a particular frequency. The thermographic images taken using infrared thermography, reveals that temperature of duckweed fronds remains unaffected upon exposure to EMR.

When the EP of *M. aquaticum* is investigated it also revealed that EMR exposure alters the standard deviation of the EP (SDEP) and the effect is varied with the frequency of the EMR. *M. aquaticum* was tested for 2, 2.5, 3.5 and 5.5 GHz EMR with a maximum field intensity of 23 – 25 V/m. The 2 GHz and 5.5 GHz exposures caused statistically significant decreases, in the SDEP. The greatest change was caused by 2.5 GHz EMR (23% increment). However, only the 2.5 GHz exposure produced a recovery of the EP after the EMR exposure, within the 1h post EMR exposure duration. The thermographic images of plants confirmed that the effect of EMR on EP is non-thermal.

As the EP of *M. aquaticum* fluctuates, we identified nanometric elongation rate fluctuation on the stem, when it was investigated using statistical interferometry technique. We named this fluctuation as nanometric elongation rate fluctuation (NERF). The SD of NERF is remaining steady when the condition of the plant is steady. When the *M. aquaticum* plants were exposed to 2 GHz EMR at a maximum electric field intensity of 23 ± 1 V/m for 1 h, plants exhibited $51 \pm 16\%$ reduction in the SD of NERF. The alteration in NERF continued for at least 2.5 h after EMR exposure and there was no significant recovery found in post-EMR NERF within the experiment period.

There are two polarizations (vertical and horizontal) of EMR are concerned based on the orientation of oscillating electric field of EMR to the earth surface. We exposed *M. aquaticum* plants to horizontal and vertical polarizations of 2GHz EMR at 26 ± 1 V/m field intensity, and examined the effects of short-duration (1.5 h) exposure to EMR on

EP, and of long-duration (48 h) exposure to EMR on plant growth parameters, pigmentation, and H₂O₂ formation. The experimental results revealed that EMR exposure has effects on the EP, pigmentation, and H₂O₂ contents of *M. aquaticum*, depending on the polarization of EMR. Exposure to horizontally polarized EMR caused electric potential to decrease after short-duration exposure, and long-duration exposure led to significantly increased H₂O₂ content. Exposure to vertically polarized EMR increased the electric potential after short-duration exposure, and long-duration exposure increased photosynthetic pigmentation significantly. However, none of the treatments altered growth parameters (shoot length, stem diameter, and node length). Thermographic images taken for 1 h continuous exposure to vertical and horizontal polarization did not indicate alteration in the temperature of the plants for any of the polarizations.

All the approaches taken to determine EMR effect on plants in present study were confirming the existence of non-thermal effect of EMR on *L. minor* and *M. aquaticum* plants. The EMR effect on plants can be depending on the frequency, exposure duration and the polarization. The techniques we used to investigate the EP and NERF are novel, and almost all the studies conducted with these techniques were carefully conducted with control experiments, to prove the adoptability of these techniques to investigate the EMR effects on plants. The field of EMR-plant interactions is very new and the pioneering researches conducted by us are encouraging further expansion of this field. It is necessary to conduct more researches to draw a general conclusion which is true for all the plants since the plant species investigated up to now are less than eight all together including the present study. Further, the existence of non-thermal effects of EMR on plants, would suggest the reconsideration of public exposure guidelines for EMR exposure, which are solely prompted based on thermal effect of EMR due to dielectric activity.