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Microwaves affect *Myriophyllum aquaticum* plants differently depending on the wave polarization

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Abstract

Previous studies on microwave exposure on plants have revealed variations in sensitivity of plants to different microwave frequencies, exposure durations, and power intensities. However, the effects of different polarizations of microwaves on plants have not been studied. Therefore, we investigated the effect of horizontally and vertically polarized 2 GHz continuous microwaves on *Myriophyllum aquaticum* plants at 1.8 W m^{-2} power density. The electric potential variation along the vascular tissues were investigated for 1.5 h and growth parameters, pigmentation, and H_2O_2 formation were studied during 48 h microwave exposure. Exposure to horizontally polarized microwaves, decreased standard deviation of electric potential variation and increased H_2O_2 content significantly. Vertically polarized microwaves increased the standard deviation of electric potential variation and photosynthetic pigments significantly. However, none of the polarizations altered growth parameters (shoot length, stem diameter, and internodal length). Thermographic images taken for 1 h continuous microwave exposure did not indicate alteration in the temperature of the plants for both vertical and horizontal polarities.

Additional key words: carotenoids, chlorophylls, dielectric activity, electric potential, emergent plant, growth parameters, non-thermal effect.

Introduction

Plants, in their natural environments, are exposed to abiotic factors, such as temperature, radiation, nutrients, and salinity, throughout their life cycle. When these factors exceed the tolerable levels, plants tend to exhibit stress responses, firstly as physiological changes and secondly as morphological changes (Ashraf and Foolad 2007 Janská *et al*. 2010). As such, presence of microwaves in the environment can be considered as an abiotic factor which modern day plants are facing (Senavirathna and Asaeda 2013). However, despite the confirmed effects, the presence of microwaves is not considered as an abiotic factor influencing plant physiology or morphology. The lack of recognition of effects of microwaves on plants is primarily due to lack of studies on this subject. On the other hand, casual observations in plants growing in natural environments do not obviously reveal the effects of microwaves; thus, awareness and understanding of these dynamics are still lacking. Therefore, further investigations of the effects of microwaves on plants are necessary.

Plants tend to exhibit elevation of stress-related hormones, reactive oxygen species (ROS), and stress related gene expressions as responses to various stresses (Wasternack and Hause 2002, Apel and Hirt 2004, Moreno *et al*. 2013). Not only photosynthesis and many other physiological pathways, but also electric potential and electric polarity change under abiotic stresses (Ray and Sinclair 1998, Ben-Gal and Shani 2002, Reyes-Diaz *et al.* 2009). The associated morphological changes are mostly identified as alterations in growth rate, leaf properties, flowering, root properties, *etc*. (Kato 1988, Gomes and Asaeda 2009). These physiological and morphological properties have also been studied under microwave exposure by various scientists (Tkalec *et al.*

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Abbreviations: EMR - electromagnetic radiation; EP - electric potential; hPol - horizontally polarized; on-EMR - during microwave exposure; post-EMR - post microwave exposure; pre-EMR - before microwave exposure; ROS - reactive oxygen species; Rx - receiving; SDEP - standard deviation of the electric potential fluctuations; Tx - transmission; vPo - vertically polarized.
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2005, Roux *et al*. 2006, Senavirathna and Asaeda 2013. 2014). They have confirmed that plants are responding physiologically and morphologically to microwaves and within a same species the responses may vary with the change of microwave frequency, incident power density, and duration of exposure. The electromagnetic radiation (EMR) consist of oscillating electric and magnetic fields travelling perpendicular to each other and perpendicular to the direction in which the energy is propagating. On the basis of the orientation of the electric field to the earth's surface, EMR is transmitted in horizontal or vertical polarization. However, knowledge on plant responses to a change of microwave polarity is currently unavailable. To address this gap in the research, we investigated the effects of vertical and horizontal polarities of microwaves on electric potential (EP), growth parameters, photosynthetic pigments, and formation of H_2O_2 in parrot feather (*Myriophyllum aquaticum*) plants. We selected *M. aquaticum* as the experimental plant based on its high apical shoot growth rate (1.8 ± 0.2 cm d^{-1}), ease of growing in nutrient solution, and a soft-woody stem that enables insertion of microelectrodes.

Materials and methods

Plants, cultivation, and treatments: Healthy, vertical, and emergent *Myriophyllum aquaticum* Verdc. shoots with partial root growth in submerged regions were cut from a *M. aquaticum* culture and the cuttings were replanted in 15 × 15 × 15-cm glass tanks. The tanks were half-filled with cleaned river sand and filled with 35 % (v/v) Hoagland's solution to a height of 5 cm above the sand. For the electric potential (EP) measurements, a cutting was planted in a random location of each tank and for other experiments, equivalent-sized shoots were selected and planted in tanks, with 3 cuttings in each tank along an edge (Fig. 1 Suppl.). Cuttings developed roots and grew to at least 10 cm above the top edge of tanks. Water level of the tanks was maintained by adding distilled water every 2 - 3 d, and once a week, the water level was amended with the 35 % Hoagland's solution. Plants were kept at a temperature of 25 - 26 ºC, a relative humidity of 70 - 75 %, a 12-h photoperiod, and an irradiance of 55 - 60 μmol m^{-2} s^{-1} (photosynthetically active radiation) using fluorescent tubes. A day before the experiments, treatment and control plants were placed inside 2 identical anechoic chambers explained in our previous study (Senavirathna and Asaeda 2014). Inside the anechoic chambers, plants were kept under same irradiance and temperature as mentioned above (Fig. 2 Suppl.).

Plants were exposed to 2 GHz continuous wave (CW) microwaves with a power density of 1.8 ± 0.1 W m^{-2} at plant surface. A transmission (Tx) antenna (2 GHz microstrip antenna) was placed facing the plants from one side at 25 cm distance (Fig. 1). Depending on the polarization (vertical or horizontal) used, the antenna was rotated 90 ° on the same plane to change from one to another (microstrip antennas transmit EMR only in a single polarity). Power densities were measured using another 2 GHz receiving (Rx) antenna at the same polarity facing parallel to the Tx antenna. Power densities were measured at 9 measuring points in a 15 cm^2 vertical plane where the plants were positioned during microwave exposure (Fig. 1). The EMR generation and the power density measurements were performed using the instrument setup explained in our previous study (Senavirathna and Asaeda 2014).

During the EP measurement, *M. aquaticum* plants were exposed continuously to microwaves for 1.5 h. For the experiments examining growth parameters, photosynthetic pigments, and H_2O_2 formation, plants were exposed to microwaves for 48 h with 1 h rest periods for instruments at 23rd h.

Electric potential along the *M. aquaticum* stem was recorded by inserting Ag/AgCl gel-stabilized glass microelectrodes. The first microelectrode was inserted 3 - 4 cm below the top of the plant, and additional microelectrode was inserted at 7 cm apart thereafter. Then the EP along the stem was recorded to a computer at a 1-s sample interval through a mV meter with a digitizer (Fig. 3 Suppl.). Detailed method for microelectrode insertion and instrument setup is described in our previous study (Senavirathna and Asaeda 2014).

Data acquisition was initiated after a 1.5 h acclimatization period following insertion of the microelectrodes Experiment continued for 4.5 h, encompassing 1.5 h each of before microwave exposure (pre-EMR), during microwave exposure (on-EMR), and post microwave exposure (post-EMR) durations. The experiment was repeated 5 times for each polarity (vertical polarity, vPol; horizontal polarity, hPol).

Growth, pigments, and H_2O_2 measurements: Plant height, internodal length, and stem diameter were quantified. Measurements were taken immediately before and after microwave exposure and then after another 48 h after microwave exposure. The height of each plant was measured as the emergent portion relative to the top edge of the tank. Internodal length and stem diameter were measured at the first node from 5 cm below the top of the plant. Plant height was measured using a millimeter scale, and internodal length and stem diameter were measured using a Vernier caliper.

After acquisition of growth parameters, plant chlorophyll *a* and *b* and total carotenoid content were quantified. Two 5-cm segments from each plant were taken from the upper and middle regions, dipped in 25 cm^3 of *N*,*N*-dimethylformamide separately and maintained for 24 h in the dark at room temperature

(20 - 24 ºC) to extract pigments. The absorption at wavelengths 663.8, 646.8, and 480 nm were determined using a *UVmini-1240* spectrophotometer (*Shimadzu*, Kyoto, Japan). Pigment content was calculated as described by Wellburn (1994).

Whole-tissue H_2O_2 content was quantified using the method described by Satterfield and Bonnell (1955) with some modifications. The 9-cm long *M. aquaticum* cuttings (fresh mass 350 - 400 mg) including the apical area were weighed, then homogenized in 30 cm^3 of cold acetone (4 ºC) and were maintained in the dark for 30 min. To determine H_2O_2 concentration, 3 cm^3 of the extract was mixed with 1 cm^3 of 0.1 % $Ti(SO_4)_2$ in 20 % H_2SO_4 and absorption was measured at 415 nm using the *UVmini-1240* spectrophotometer. The concentration of H_2O_2 in the solution was obtained from a calibration curve.

The experiments were repeated 3 times for both polarities (vPol and hPol) with 3 plants in each repetition, and controls were carried out simultaneously.

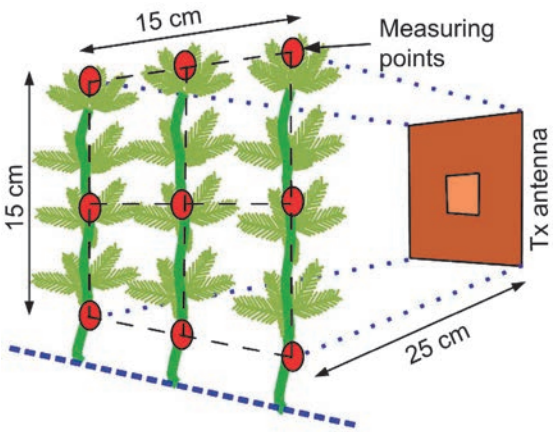

Fig. 1. Antenna orientation toward plants and power density measured at vertical plane. Plants were placed 25 cm away from the transmission antenna (Tx antenna - 2 GHz microstrip antenna). Radiated power density measured at 9 points (marked ovals) in the 15 × 15 cm vertical plane.

Thermographic images were obtained to determine temperature changes in *M. aquaticum* plants after 1-h continuous exposure to 2 GHz microwaves with power density of 1.8 ± 0.1 W m^{-2}. Images were taken before microwave exposure and upon completion of 1-h continuous microwave exposure for both hPol and vPol. Room temperature was maintained at 25 - 26 ºC. Thermal images were obtained using an infrared thermographic camera with thermal sensitivity of 0.08 °C (*InfReC Thermo GEAR G120*, *NEC*, Tokyo, Japan). The emissivity of the plant was considered as 0.95.

Data analysis: The EP of *M. aquaticum* is not linear over time. To create a linear signal, the recorded EP was modified by calculating the differences between each adjacent data point (*i.e.*, subtracting the previous value from the subsequent value and plotting against time). The modified signal consists of rapid and continues EP fluctuations. Then the standard deviation of these EP fluctuations (SDEP) were calculated separately for the pre-EMR, on-EMR, and post-EMR durations. The complete procedure for creating linear signal and SDEP calculation is described in our previous study (Senavirathna and Asaeda 2014; Fig. 4 Suppl.). The SDEPs of three durations (pre-EMR, on-EMR and post-EMR) were normalized by dividing the SDEP of pre-EMR. The normalized SDEPs were then compared using 2-independent-sample *t*-tests at the $P < 0.05$. To prevent possible misinterpretations due to the EP signal being manipulated by the electric field of the microwaves, the EP data of on-EMR were not discussed in the present experiment.

The growth parameters were normalized by dividing parameters obtained on the completion of 48 h microwave exposure and 48 h after microwave exposure from initial measurements. Since the data were not normally distributed (Levene's test for equality of variances, $P > 0.05$), the Mann-Whitney U-test was used to compare data, considering $P < 0.05$ as significant.

The chlorophyll *a* and *b*, total chlorophyll, total carotenoid, chlorophyll *a*:*b* ratios, and H_2O_2 content were also not normally distributed (Levene's test for equality of variances, $P > 0.05$) and were compared for significant results using the Mann-Whitney U-test (considering $P < 0.05$ as significant). Pigment content was significantly different between two 5-cm segments taken from the same plant in both the control and microwave-exposure experiments (Mann–Whitney *U*-test, $P < 0.01$). Therefore, the 2 segments were analyzed separately. All statistical analyses were performed using *SPSS 16.0* (*International Business Machines*, Armonk, NY, USA).

Thermographic images were manually analyzed for differences in temperature before, and upon completion of 1 h continuous microwave exposure.

Results

The horizontally polarized 2 GHz microwave exposure reduced SDEP significantly. The post-EMR SDEP was 20 % less than pre-EMR SDEP. The vertically polarized microwave exposure caused SDEP to increase by 25 % during post-EMR. The increased SDEP of post-EMR was significant over the pre-EMR (Fig. 2).

The plant heights, internodal lengths, and stem diameters were not altered significantly during exposure to vPol or hPol for 48 h, or after 48 h microwave exposure. The average plant length, internodal length, and stem diameter increased by 49, 20, and 8 %, respectively, during the hPol exposure, and after 48 h microwave exposure these parameters increased by 24, 3, and 7 %, respectively. During the vPol exposure, plant

length, internodal length, and stem diameter increased by 45, 20, and 10 %, respectively, and 48 h after exposure to vPol, plant length, internodal length, and stem diameter increased by 23, 5, and 6 %, respectively. In the control plants, plant length, internodal length, and stem diameter increased by 44, 19, and 11 %, respectively, during the first 48 h, while after the next 48 h these parameters increased by 24, 6, and 5 %, respectively (Table 1).

Chlorophyll *a* and *b*, total chlorophyll, total carotenoids, and chlorophyll *a*:*b* ratio in the lower 5-cm segments of *M. aquaticum* plants did not differ significantly between microwave-treated and control plants for either hPol or vPol exposure. In the upper 5 cm segments exposed to hPol, none of the above-mentioned parameters were altered compared to the control. However, chlorophyll *a* and *b*, total chlorophyll, and total carotenoid content in the upper 5 cm segment exposed to vPol changed significantly from the control (by 28, 13, 24, and 20 %, respectively), while the chlorophyll *a*:*b* ratio did not change (Table 2).

Exposure to hPol for 48 h caused a significant increase in plant H_2O_2 content. The H_2O_2 content in plants exposed to hPol and in control plants were 571 ± 13 and 534 ± 21 nmol g^{-1}(f.m.), respectively. However, exposure to vPol did not change H_2O_2 content; plants exposed to vPol and control plants contained an average of 542 ± 13 and 542 ± 19 nmol g^{-1} (f.m.) H_2O_2, respectively (Fig. 3).

The thermographic images taken for both hPol and vPol exposure revealed that 1 h continuous exposure to microwave caused no temperature change in *M. aquaticum* plants. The average temperature in the canopy area was 25.2 ± 0.1 ºC prior to hPol exposure and 25.1 ± 0.1 ºC after 1 h continuous hPol exposure; the respective values for exposure to vPol were 24.9 ± 0.1 and 25.1 ± 0.2 ºC (Fig. 5 Suppl.).

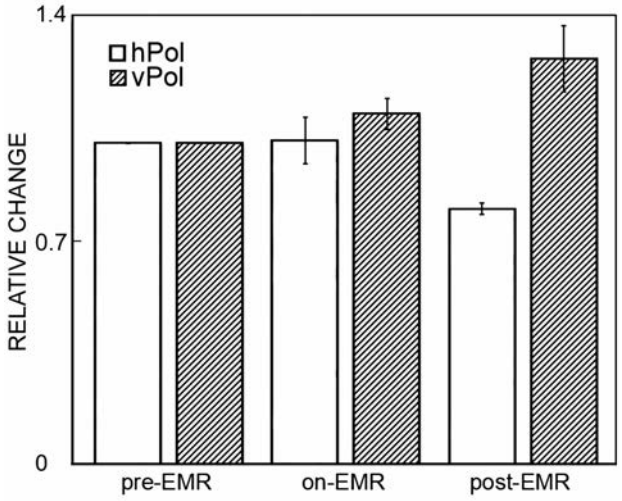

Fig. 2. Relative changes of standard deviation of the electric potential fluctuations (SDEP). The labels pre-EMR, on-EMR, and post-EMR on the x-axis represent before microwave exposure, during microwave exposure and after microwave exposure, respectively. The hPol and vPol indicate horizontally polarized and vertically polarized microwave exposures. Means ± SDs, *n* = 5.

Table 1. Plant length [length; cm], internodal length [node; cm], and stem diameter [stem; mm] of *Myriophyllum. aquaticum* plants exposed to horizontally (hPol) and vertically (vPol) polarized 2 GHz microwaves. Means ± SDs, *n* = 9. Parameters of before microwave exposure, during 48 h microwave exposure, and 48 h after microwave exposure are not significantly different.

	Before microwave exposure			During 48-h microwave exposure			48-h after microwave exposure		
	length	node	stem	length	node	stem	length	node	stem
hPol	8.12±1.55	1.56±0.16	2.08±0.22	12.05±2.17	1.86±0.20	2.25±0.21	14.85±2.25	1.91±0.23	2.40±0.23
vPol	9.06±1.90	1.59±0.22	1.96±0.15	12.94±2.08	1.91±0.24	2.14±0.18	15.87±2.07	2.00±0.26	2.27±0.18
Control	8.47±1.90	1.54±0.25	2.02±0.16	12.05±1.88	1.84±0.26	2.24±0.14	14.86±2.24	1.96±0.27	2.34±0.14

Table 2. Effects of horizontally (hPol) and vertically (vPol) polarized 2 GHz microwaves on photosynthetic pigments [µg g^{-1}(f.m.)] in upper and lower 5 cm cuttings of *Myriophyllum. aquaticum* plants. Means ± SDs, *n* = 9, * - significantly different from control ($P < 0.05$).

		Chl *a*	Chl *b*	Total Chl	Chl *a/b*	Total Car
Upper	hPol	1053±88	359±66	1412±143	2.99±0.44	230±18
	vPol	1341±83*	443±28*	1785±110*	3.03±0.03	272±24*
	control	1050±192	391±71	1441±236	2.75±0.57	227±38
Lower	hPol	616±45	189±43	806±82	3.36±0.58	136±9
	vPol	653±112	236±20	889±128	2.27±0.36	142±18
	control	560±117	216±94	777±190	2.83±0.71	122±20

Discussion

The electric field of microwaves can increase the temperature of biological tissues. Heat generation in the tissues occurs by vibration of water molecules (dipolar molecules) and charged ions (positively or negatively charged), due to dielectric activity resulted from the rapidly oscillating electric field of microwaves (Jacob *et al.* 1995). However, in the present study, thermographic images revealed that microwave exposure caused no significant impact on the temperature of plants. Although there was no heat incident, dielectric activity could be presented at low intensity, which could affect the physiology of plants. Considering *M. aquaticum*, exposure to vPol should vibrate water molecules and charged ions along the plant stem, while exposure to hPol should cause the vibration phenomenon to occur across the stem (Fig. 4). Therefore, these 2 polarizations can have different effects on plants. The observations of decreased SDEP under hPol exposure and increased SDEP under vPol exposure, and of significantly increased pigment content only for vPol, and of significantly increased H_2O_2 content only for hPol exposure, demonstrate the existence of the differential effects of microwave polarization on plants.

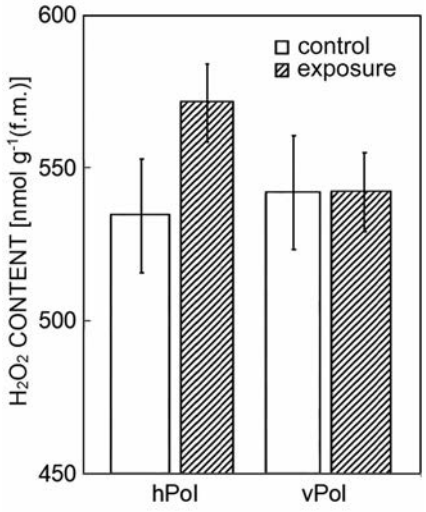

Fig. 3. Hydrogen peroxide content of *Myriophyllum aquaticum* control plants and plants exposed to horizontally (hPol) and vertically (vPol) polarized 2 GHz microwaves. Means ± SD, $n = 9$.

Exposure to magnetic fields and electric fields of alternating current causes a cellular influx of Ca^{2+} ions in hepatoma and T lymphocyte cells of humans (Lindströum *et al.* 1993, Cho *et al*. 1999). Further, Ca^{2+}-binding messenger proteins (calmodulin) has been activated in very low intensity magnetic fields (20 μT; Liboff *et al*. 2003) and calmodulin was accumulated in tomato plants exposed to low intensity 900 MHz EMR (Roux *et al*. 2006). This lead to an increased concentration of Ca^{2+} inside cells and a decrease in the extracellular space. The alteration of Ca^{2+} balance should alter the EP in plants, since the EP is maintained by the Ca^{2+}, Cl^-, and K^+ ion balance between the intra and extracellular spaces (Felle and Zimmermann 2007, Fromm and Lautner 2007). The increased H_2O_2 content in *M. aquaticum* plants under hPol exposure is consistent with the above mentioned Ca^{2+} influx mechanism, since it was reported that an influx of Ca^{2+} increases the generation of H_2O_2 inside cells (Yang 2002). Therefore, the decreased EP and increased H_2O_2 observed in plants exposed to hPol are consistent with the above mentioned mechanisms.

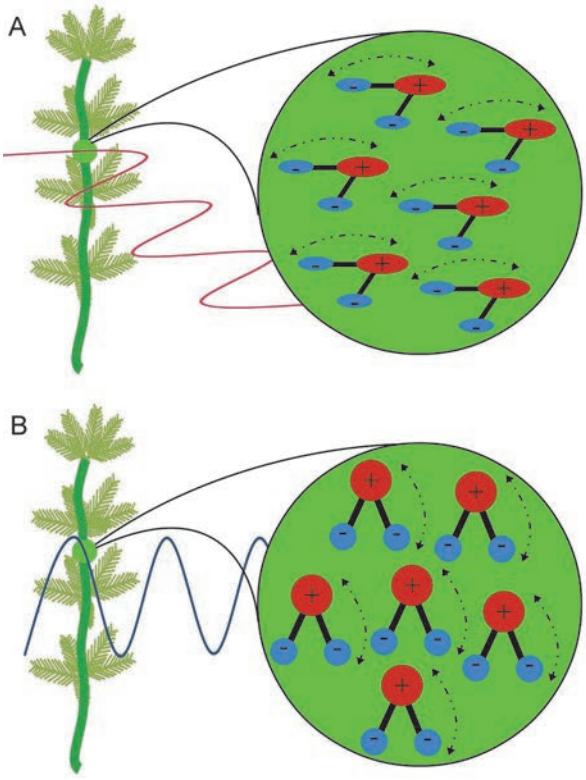

Fig. 4. Vibration of water molecules due to microwave exposure. *A* - horizontally polarized microwave exposure caused water molecules to align and to rotate left and right in the horizontal plane. *B* - vertically polarized microwaves caused water molecules to align along the vertical plane and to rotate up and down.

The accumulation of H_2O_2 in plants due to short-duration microwave exposure was reported by Tkalec *et al*. (2007) and Sharma *et al.* (2009); however, these studies did not account for polarizations. In the present study, we confirmed that hPol exposure causes oxidative stress in plants. Excess formation of H_2O_2 in plants generally has a negative influence on plant growth and development (Gechev and Hille 2005, Cheeseman 2007, Petrov and Van Breusegem 2012). Although exposure to hPol caused significant increases in H_2O_2 content in the present study, there was no significant impact on the

growth and development of *M. aquaticum* plants. The increased H_2O_2 content (7 % greater than in the control) may be within *M. aquaticum* tolerance levels.

When plants were exposed to static (10 mT) and 0.01 to 63000 Hz pulsed (10 - 100 mT) magnetic flux density, photosynthetic pigment content increased (Dhawi and Al-Khayri 2009, Răcuciu 2012). Even with the relatively low incident magnetic flux density (approximately 0.025 μT) applied in the present study, vPol exposure increased photosynthetic pigment content whereas hPol exposure did not effect the pigment content. Therefore, exposure to perpendicular magnetic fields seems to have a greater influence on *M. aquaticum* plants. Previous studies on effects of microwaves on photosynthetic pigmentation of *Zea mays* seedlings explain that stimulation or inhibition of photosynthetic pigmentation depends on the frequency of exposure (Răcuciu and Miclăuş 2007, Ursache *et al.* 2007). However, these studies did not consider the effect of polarizations. Therefore, the present study confirms that even within the same frequency, the effect of microwaves on pigmentation could depend on the polarity which plants are exposed to.

Conclusion

Present findings provide evidence of different responses in plants based on the polarity of the microwaves to which they are exposed. In addition, our results suggest that perpendicular exposure to each field (magnetic and electric) has a stronger influence on plants than parallel exposure. Therefore, the influence of microwaves on plants in the environment is highly complex, and the type of response that can be expected will differ from one location to another, even within the same species. Further, the maximum exposure guidelines for microwave range were developed considering the thermal effect that could be caused by dielectric heating of biological tissues. However, the present study confirms non-thermal effects of microwaves on plants, calling into question the validity of these exposure guidelines.

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