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Stimulatory effect of exposure to low-power-density 2.45 GHz microwaves on *Arabidopsis thaliana* seedlings in vitro

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Abstract

Communication technologies are moving toward higher microwave frequencies and bandwidths to satisfy the growing demand for high data rates. The concern about the possible effects of microwaves on plants and animals has increased recently. There is still uncertainty concerning the effects of microwaves on plants. The present study was conducted to investigate the effect of industrial, scientific and medical (ISM) radio band microwaves on seedlings and seeds of *Arabidopsis thaliana* (L.). In vitro growing *A. thaliana* wild-type seedlings and seeds were exposed to 2.45 GHz continuous-wave microwaves at a power flux density of $1.0 \pm 0.1 \ W \ m^{-2}$ for 48 h. Microwave exposure increased the hydrogen peroxide content, photosynthetic pigments, nonphotochemical quenching and fluorescence of the seedlings, while peroxidase activity and F_v/F_m values were unchanged. Anthocyanin and malondialdehyde were decreased. Seed germination rate, fresh weight and photosynthetic pigment contents of 10-day-old seedlings obtained from microwaves exposed seeds remained unchanged. Results confirmed the inexistence of oxidative stress but a stimulatory effect of microwave on *A. thaliana* seedlings. The increased hydrogen peroxide content and nonphotochemical quenching suggest acceptance of extra photon energy and a portion of the excess captured photon passing through the photosystem, while a portion of energy dissipated as heat.

Keywords Chlorophyll fluorescence · Pigmentation · Physiochemical · Radiofrequency electromagnetic radiation · Short-duration exposure

1 Introduction

Numerous studies have focused on the effects of microwaves on human health which have confirmed that exposure guidelines, which are solely based on thermal effect, are no longer providing reliable protection to humans (Senavirathna and Asaeda 2014). On the other hand, plants, which are currently not considered for protection against microwaves, are vulnerable to the consequences of exposure. Currently, a number of studies have confirmed that microwaves can also be an abiotic factor for plant health. As per the present findings, plants' responses to microwaves range from phenotypic

and physiological parameters to genotypic parameters (Vian et al. 2006; Tkalec et al. 2007; Halgamuge et al. 2015). However, the response of plants to microwaves is specific to the microwave frequency, power density, polarity and exposure duration; in some observations, the exhibited responses were both negative and positive stimulatory behaviors (Răcuciu and Miclăuş 2007; Roux et al. 2008; Senavirathna and Asaeda 2017).

Each of the growth stages of a plant is critical for a plant to occupy its environment and complete its life cycle successfully. The seed stage can be influenced by mechanical and chemical properties, as well as temperature, and can cause changes in the germination rate and physiology and morphology of the seedlings after germination (Pe et al. 1975; Jennings and Saltveit 1994; Posmyk et al. 2009). Particularly, the seedlings and early growth stages of plants are highly vulnerable to external influences. Stress factors such as light, temperature, water and salinity can all affect seedlings (Duysen and Freeman 1974; Von Arnim and Deng 1996; DeRose-Wilson and Gaut 2011; Rodríguez et al. 2015); these effects can be distinguishable in

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the development and mature stages of plants, even when the stress is no longer present (Techawongstien et al. 1992; Johkan et al. 2010).

There are few existing studies on the effect of microwaves on the seedling stages of plants, and most of the studies have confirmed the effects of microwaves on the seedlings. Among them, Scialabba and Tamburello (2002) confirmed the decreased growth rate of radish (*Raphanus sativus* L.) seedlings under low power densities; however, this result was conducted with currently environmentally insignificant frequencies (10.5 and 12.5 GHz), though there is the potential for them to become prominent with the development of 5G technologies. Three-week-old tomato plants (*Lycopersicon esculentum* Mill. cv. VFN-8) exposed to 0.9 GHz microwaves at a low power density exhibited the accumulation of a wound-related transcription factor (LebZIP1 mRNA; Vian et al. 2006). Research conducted by Tafforeau et al. (2006) also confirmed the modified expression of a proteome resembling cold shock in *Arabidopsis* seedlings (strain not specified) exposed to irradiation from a GSM mobile phone for 2 h (frequency and power density unknown). A morphological study conducted by Skiles (2006) on the exposure of mature Alfalfa (*Medicago sativa* L.) 2.45 GHz microwaves at 5–12 W m^{-2} power flux density resulted insignificant effects on the plants. Therefore, with both effected and non-effected observations, there is still uncertainty present in the effects of microwaves on plants.

Communication technologies are moving toward higher microwave frequencies and bandwidths to satisfy the growing demand for high data rates. The 2.45 GHz microwaves frequency which is an industrial, scientific and medical (ISM) radio band (2.4–2.5 GHz with a center band of 2.45 GHz) was utilized in almost all the Wi-Fi and wireless consumer electronics. In order to investigate the effect of ISM band microwaves on plants, the present study was conducted to investigate the effect of 2.45 GHz microwave on *Arabidopsis thaliana* L. (Columbia) wild-type seed germination and seedling biochemical and physiological parameters. This research was conducted at a power flux density of 1.0 ± 0.1 W m^{-2} and microwave frequency of 2.45 GHz, which is well below the maximum human exposure limits stipulated in the International Commission on Non-Ionizing Radiation Protection guidelines (10 W m^{-2}; Ziegelberger 2009).

2 Method

2.1 Seedling preparation – *A. thaliana* wild-type seedlings were prepared in vitro in disposable polystyrene Petri dishes with Phytagel medium. Approximately 35–40 seeds were placed in a single Petri dish (Fig. 1). After placing the seeds, Petri dishes were closed and sealed to prevent contamination. Plates were kept in the dark at 4 °C for 3 days for cold stratification. Then, the Petri dishes were maintained under a light intensity of 80–85 µmol m^{-2} s^{-1} with 18:8 h light/dark period at 25 ± 1.0 °C in a temperature-controlled room. Illumination was applied with LED lights with a color rendering index of 80 (Model LT-NLD85L-HN; OHM Electric INC, Japan). After 6 more days, the Petri dishes were transferred to two identical anechoic chambers (microwave treatment and control), in one of which the microwave exposure was conducted. The light intensity, light duration and temperature were maintained throughout the experimental period. Seedlings were kept for 4 days inside the anechoic chambers for acclimation.

Fig. 1 **a** *Arabidopsis thaliana* wild-type seedling-containing Petri dishes inside the microwave treatment anechoic chamber with a 2.45 GHz microstrip antenna installed from the top. **b** *A. thaliana* wild-type seedlings containing Petri dishes inside the control anechoic chamber
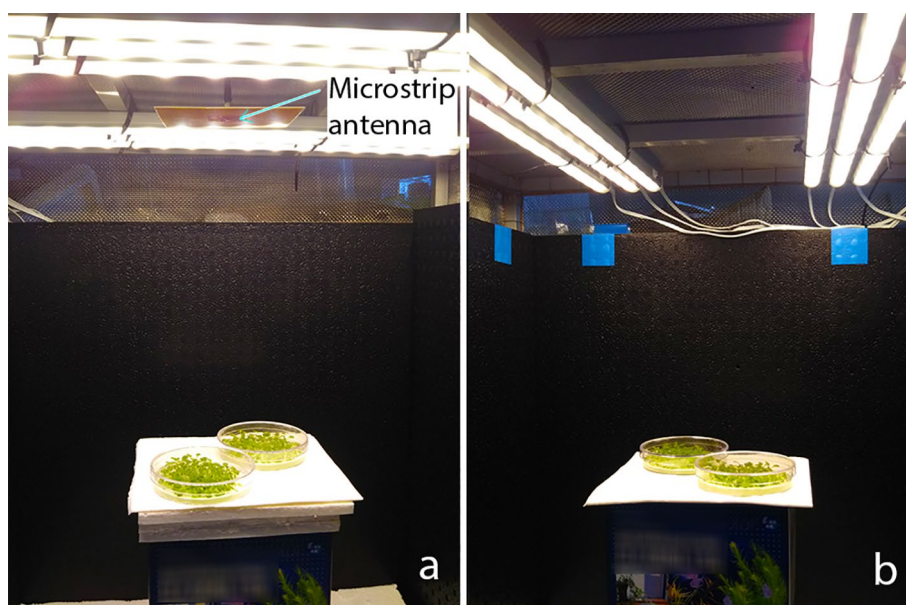
2.2 Anechoic chambers – Anechoic chambers were used to isolate the seedlings from the influence of external electromagnetic wave sources (i.e., mobile phone base stations, Wi-Fi base stations and television and radio broadcasts) and to prevent possible interference from communication equipment. The anechoic chambers layered with EMR absorbing ferried containing foams (PFP 30, Riken, Japan), which were described in our previous study (Senavirathna et al. 2014), were modified for the present study (Supplementary materials 1). The anechoic chambers were lit with six 57 cm straight LED lights (Model LT-NLD85L-HN, OHM Electric INC, Japan). To remove the inside heat, chambers were equipped with an externally fixed exhaust fan on the top (Type ASEN104519; Panasonic Industrial Devices SUNX Co., Ltd, Aichi, Japan).

2.3 Microwave exposure – The Arabidopsis seeds and seedlings were exposed to 2.45 GHz continuous-wave microwaves for 48 h from a top-hanging microstrip antenna (Fig. 1). The power density at the seedlings was measured in random spots of a horizontal plane where the seedlings were kept and maintained at an average 1.0 ± 0.1 W m^{-2}. The exposure lasted for 24 h, after which the microwave transmission system was switched off for 1 h (to rest the instruments). On the completion of the 1-h break, the microwave transmission system was switched on and the treatments were continued for another 24 h. The microwave transmission and the power density measurements were performed with the systems described previously (Senavirathna et al. 2014; Supplementary materials 2). The microwave transmission side contains a signal generator (8350 B oscillator with 83592B RF plugging, Hewlett Packard, Santa Rosa, CA, USA), a linear amplifier (ZVE-3W-83+, Mini-Circuits, New York, USA). The power density measuring system contains a power meter (ML 2472A, Anritsu, Kanagawa, Japan) via a power sensor (MA 2471A, Anritsu).

2.4 Germination test – Arabidopsis seeds collected from a single parent population were kept in 2.5-mL plastic microcentrifuge tubes under the microwave transmission antenna and treated with 2.45 GHz microwaves for 48 h at 1.0 ± 0.1 W m^{-2} power density following the same exposure procedure as that for the seedlings. Simultaneously, the control samples were maintained in the control chamber. After the exposure, control and treated seeds were subjected to the usual germination procedure of *Arabidopsis* seedlings, described before. After cold stratification, the gel plates were kept inside the anechoic chambers without microwave exposure. After 10 days, the number of germinated seeds was counted, and the photosynthetic pigment contents of the seedlings were measured.

2.5 Chemical analysis – Seedlings were subjected to chemical extraction soon after the completion of the microwave treatment. Approximately 100 mg from each of the microwave-treated and control samples was weighed out and crushed in liquid nitrogen. The samples were then further crushed in the presence of 100 μL of 2% polyvinylpyrrolidone (PVP) and extracted into 5 mL of ice-cold 0.05 M phosphate buffer (pH 6.0). The extraction was cold centrifuged (4 °C) at 2500 g for 10 min, and the supernatant was collected and stored at -80 °C until further analysis (Atapaththu and Asaeda 2015).

The H_2O_2 contents of seedlings were measured as per the method described by Satterfield and Bonnell (1955) with modifications. A 750 μL of the aliquot was reacted with 2.5 mL of 0.1% titanium sulfate in 20% (v/v) H_2SO_4. The intensity of the resulting pale-yellow color was measured spectrophotometrically (UV 1280, Shimadzu, Nagoya, Japan) at the 410 nm wavelength. H_2O_2 concentrations were estimated using a standard curve for H_2O_2, and the results were presented in μmol g^{-1} FW.

The peroxidase activity was measured using guaiacol as the hydrogen donor. The enzyme extract (100 μL) was subjected to reaction with 40 μL of 30 mM H_2O_2 in the presence of 50 μL of 25 mM guaiacol. The reaction was initiated upon the addition of enzyme extract into the reaction medium. The resulting color development was determined spectrophotometrically (UV 1280) at a 420 nm wavelength. Absorption was recorded every 10 s for 3 min. Based on the rate of color development, POD activity was determined using the extinction coefficient of 26.6 mM^{-1} cm^{-1}. The POD activity was expressed as μmol min^{-1} g^{-1} FW (MacAdam et al. 1992).

The lipid peroxidation was measured as the malondialdehyde (MDA) content of the whole seedling according to the method of Zhang and Huang (2016) with modifications. Seedlings (50 mg) were crushed to powder with liquid nitrogen and mixed with 0.1% trichloroacetic acid (TCA) and mixed well. Then, 4 mL of 20% TCA containing 0.5% thiobarbituric acid (TBA) was added, and the mixture was boiled in a water bath for 30 min and quickly cooled in ice. The mixture was centrifuged at 4 °C at 2500 g for 10 min. The optical absorption of the supernatant was spectrophotometrically (UV 1280) measured at 600 nm, 532 nm and 450 nm. The MDA content was calculated (μmol/gFW) through the following equation:

$$MDA = 6.45(A_{532} - A_{600}) - (0.56 \times A_{450})$$

The seedlings were subjected to photosynthetic pigment extraction soon after the completion of microwave exposure or completion of the growth period (control).

The photosynthetic pigment contents were estimated by extracting the pigments from *N*,*N*-dimethylformamide. Pigments (approximately 50 mg) of whole seedlings were extracted into 5 mL of *N*,*N*-dimethylformamide (seedlings were kept in *N*,*N*-dimethylformamide for 24 h under darkness at room temperature). The optical absorption of the extraction was measured at wavelengths of 664, 647 and 480 nm using the UV 1280 spectrophotometer. The chlorophyll *a* (Chl *a*), *b* (Chl *b*) and total carotenoid (Car) contents were calculated using the equation provided by Wellburn ([1994](#)) and expressed as µg g^{-1} FW.

The anthocyanin content of the seedlings was measured spectrophotometrically as per the method described in Nakata et al. ([2013](#)) with modifications. Approximately 50 mg of whole seedlings was crushed to a powder in liquid nitrogen. The pigments were extracted using 2 mL of extraction buffer containing 45% methanol and 5% acetic acid in water. After centrifuging at 3500 rpm for 15 min at 20 °C, the supernatant was collected. The optical absorption was measured spectrophotometrically at 637 nm and 530 nm. The anthocyanin content was expressed in anthocyanin units, which is one anthocyanin unit equal to one absorbance unit in 1 mL of extraction solution (Teng et al. [2005](#)):

$$\text{units g}^{-1}\text{ FW} = \left[A_{530} - \left(0.25 \times A_{637}\right)\right] \times M$$

where M is the added extraction buffer weight divided by the sample weight.

**2.6 Chlorophyll fluorescence and nonphotochemical quenching** – Seedlings were subject to chlorophyll fluorescence (ChF) and nonphotochemical quenching (NPQ) measurements just after the microwave exposure (15-min dark adaptation was applied while the microwave treatment is continued). The maximum PSII quantum yield (F_v/F_m), instantaneous PSII quantum yield during light adaptation (QP_L), coefficient of photochemical quenching during dark relaxation (QP_D), instantaneous fluorescence during light adaptation (Ft_L), steady-state fluorescence in light (Ft_S), steady-state nonphotochemical quenching (NPQ_S), instantaneous nonphotochemical quenching during dark relaxation (NPQ_D) and instantaneous nonphotochemical quenching during light adaptation (NPQ_L) were considered. The ChF and NPQ parameters of all the control and treatment seedlings (approximately 40 seedlings) were measured and averaged using a ChF imagine technique (Handy FluorCam, FC 1000-H; Photon System Technology, Brno, Czech Republic) with default quenching analysis protocol supplied with the software FluorCam 6.0 ver. 0.5.2.190 (Supplementary material 3). Average values of fluorescence and quenching parameters were obtained with the automatic selection

settings of the software provided with the Handy FluorCam instrument (FluorCam 6.0 ver. 0.5.2.19).

2.7 Data analysis – All the descriptive statistics and data visualizations were performed using the inbuilt functions of Microsoft Excel 2016. Significant differences between control and microwave-exposed groups were tested using Student's *t* test, assuming equality of variance using IMB SPSS Statistics for Windows, Version 25 (IBM Corp., Armonk, NY, USA).

3 Results

The oxidative stress of the seedlings was increased significantly after 48 h of microwave exposure (treatment). The increase in tissue H_2O_2 content was approximately 2.5 times that of the control samples, which was 2.93 ± 1.19
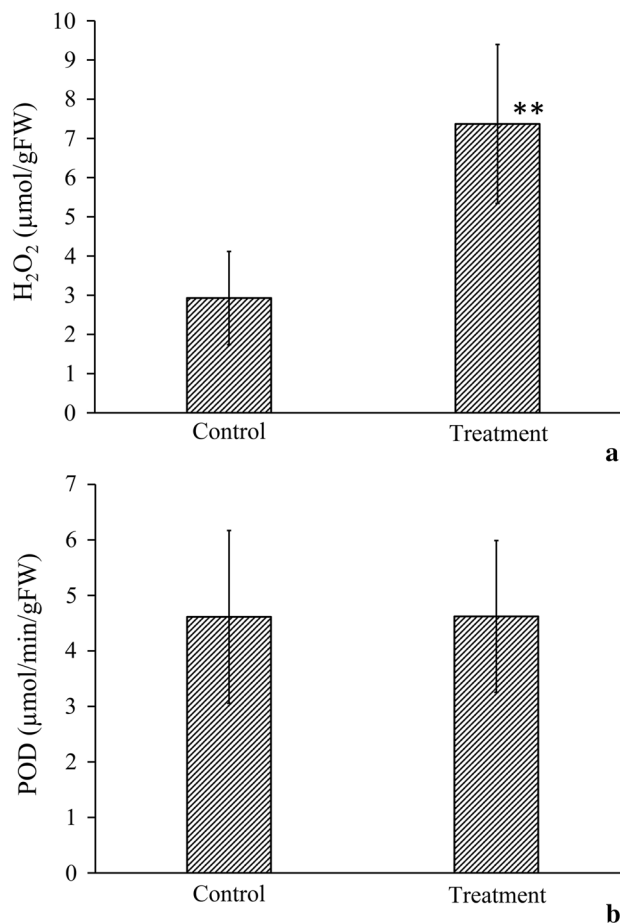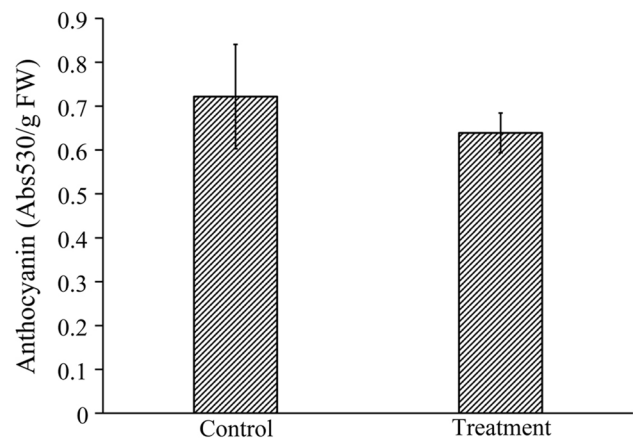

Fig. 2 **a** H_2O_2 content of 12-day-old *Arabidopsis thaliana* wild-type seedlings under control and 48-h microwave exposure (treatment) conditions. **b** POD activity of the same seedlings under control and treatment conditions. The '**' represents the significant difference between control and treatment ($P < 0.01$). Error bars represent the standard deviation
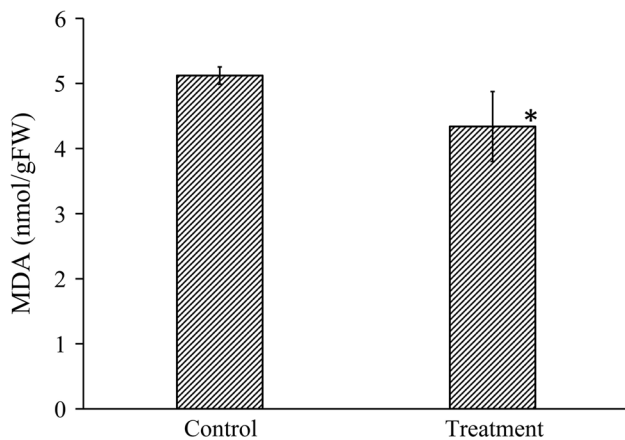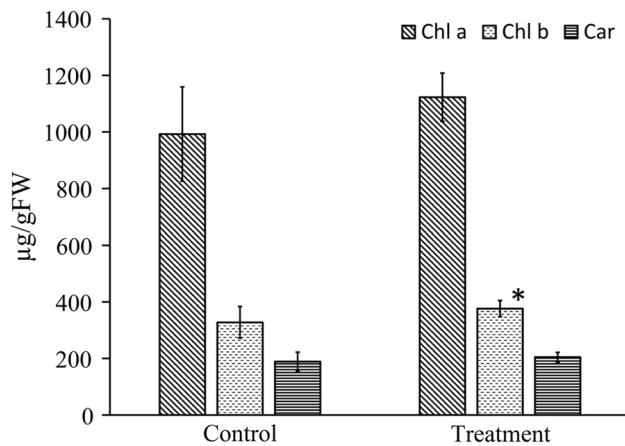
Fig. 3 MDA content of 12-day-old *Arabidopsis thaliana* wild-type seedlings under control and 48-h microwave exposure conditions. Error bars represent the standard deviation
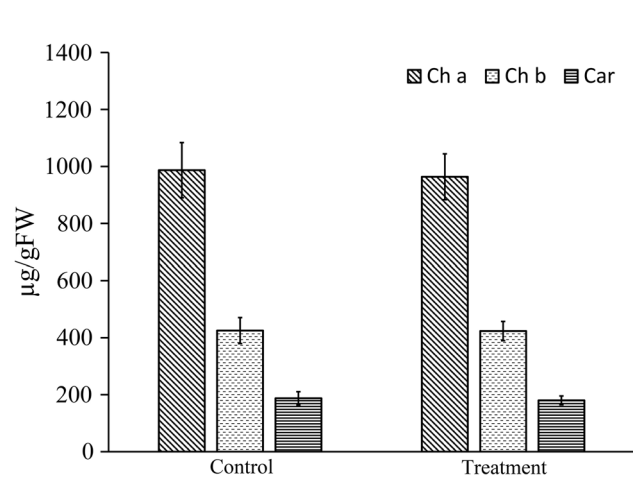

Fig. 5 Anthocyanin content of 12-day-old *Arabidopsis thaliana* seedlings under control and 48-h microwave exposure conditions. The '*' represents the significant difference between control and treatment ($P < 0.05$). Error bars represent the standard deviation

Fig. 4 Photosynthetic pigment content (chlorophyll a, Chl a; chlorophyll b, Chl b; total carotenoids (Car) of 12-day-old *Arabidopsis thaliana* seedlings under control and 48-h microwave exposure (Treatment) conditions. The '*' represents the significant difference between control and treatment ($P < 0.05$). Error bars represent the standard deviation

Fig. 6 Photosynthetic pigment contents (chlorophyll a, Chl a; chlorophyll b, Chl b; total carotenoids (Car)of 10-day-old *Arabidopsis thaliana* seedlings of control 48-h microwave-treated seeds (treatment). Error bars represent the standard deviation

and 7.36 ± 2.03 μmol g^{-1} FW for the control and treatment samples, respectively (Fig. 2a, *t* test, $P < 0.01$). However, the peroxidase activity of the control and the treatment samples was unchanged at 4.62 ± 1.56 and 4.61 ± 1.36 nmol g^{-1} FW, respectively (Fig. 2b). The MDA content of the treatment was significantly less (*t* test, $P < 0.05$) than that of the control, at 5.12 ± 0.13 and 4.33 ± 0.53 nmol g^{-1} FW, respectively (Fig. 3).

The average Chl *a* and *b* contents were higher in the treatment samples than in control. The Chl *a* and *b* contents of the control samples were 1037 ± 105 and 343 ± 35 μg g^{-1} FW, while those of the treated samples were 1123 ± 85 and 376 ± 29 μg g^{-1} FW, respectively. The Car contents of the control and treatment samples were 197 ± 26 and

204 ± 17 μg g^{-1} FW, respectively (Fig. 4). Among the photosynthetic pigments, the Chl *b* contents were significantly different (*t* test, $P < 0.05$). The Chl *a*/*b* ratios of the control and the treatment samples were 3.02 ± 0.02 and 2.98 ± 0.02, respectively, and showed a significant difference (*t* test, $P < 0.01$). The anthocyanin contents of treatment samples exhibited a statistically insignificant reduction than the control (independent sample *t* test, $P > 0.05$). The anthocyanin units (Abs 530 g^{-1} FW) were 0.72 ± 0.11 and 0.63 ± 0.04 for the control and treatment samples, respectively (Fig. 5).

The Chl *a*, Chl *b*, and Car contents of the 10-day-old seedlings of microwave-exposed seeds remained unchanged (*t* test, $P > 0.05$). The Chl *a* and *b* contents of the control samples were 987 ± 97 and 425 ± 46 μg g^{-1} FW, while those of the treatment samples were 964 ± 80

and 423 ± 34 μg g^{-1} FW, respectively. The Car contents of the control and treatment samples were 187 ± 23 and 180 ± 15 μg g^{-1} FW, respectively (Fig. 6). The Chl a/b ratios of the control and treatment samples were 2.28 ± 0.03 and 2.32 ± 0.03. The germination test registered a 98% success rate for both the treatment and control samples.

The values and the images of chlorophyll fluorescence and quenching parameters are shown in Table 1 and Fig. 7. Accordingly, the NPQ values in light (NPQ_L), dark (NPQ_D) and a steady-state of light (NPQ_S) were increased significantly by 25.5%, 115%, and 27.9%, respectively, in the microwave-exposed samples compared to in control (t test, $P < 0.01$ for all three parameters). The fluorescence values in the light state (Ft_L) and steady state of light (Ft_S) were significantly reduced by 8.1% and 8.8%, respectively, in the microwave-exposed samples (t test, $P < 0.05$ for Ft_L and $P < 0.05$ for Ft_S). However, the maximum quantum yield (F_v/F_m), photochemical quenching in light (QP_L) and photochemical quenching in dark (QP_D) were statistically unchanged (t test, $P > 0.05$).

4 Discussion

The microwave irradiation applied in the present experiment, 1.0 ± 0.1 W m^{-2}, is well within the exposure guidelines for the 2.45 GHz frequency range, which is 10 W m^{-2} for public exposure (ICNIRP 1998). The present experiment proved the existence of exposure effects on seedlings, as some of the measured parameters were altered compared to those in control. However, although previous studies have reported stress or negative impacts (Senavirathna and Asaeda 2014), the present findings showed a somewhat stimulatory effect of microwaves on *Arabidopsis* seedlings, though the H_2O_2 level was increased compared to that of the control.

The increased H_2O_2 level was 2.5-fold that of the control, which is a significant increase; however, the unchanged POD activity suggested that the H_2O_2 level in the treatment plants may have remained within the

Table 1 Nonphotochemical quenching and chlorophyll fluorescence parameters of 12-day-old *Arabidopsis thaliana* seedlings under control and 48-h microwave exposure conditions

NPQ_S**	NPQ_D**	NPQ_L**	F_v/F_m	QP_L	QP_D	Ft_L*	Ft_S**
Control							
0.55 ± 0.07	0.15 ± 0.03	0.55 ± 0.05	0.81 ± 0.02	0.48 ± 0.04	0.92 ± 0.01	28.10 ± 2.54	28.69 ± 2.49
Treatment							
0.70 ± 0.09	0.31 ± 0.03	0.72 ± 0.07	0.81 ± 0.01	0.49 ± 0.02	0.94 ± 0.02	25.83 ± 2.21	26.16 ± 2.83

Control, seedlings without microwave exposure; treatment, 48-h microwave-exposed seedlings; NPQ_S, steady-state nonphotochemical quenching; NPQ_D, instantaneous nonphotochemical quenching during dark relaxation; NPQ_L, instantaneous nonphotochemical quenching during light adaptation; F_v/F_m, maximum PSII quantum yield; QP_L, instantaneous PSII quantum yield during light adaptation; QP_D, coefficient of photochemical quenching during dark relaxation; Ft_L, instantaneous fluorescence during light adaptation; Ft_S, steady-state fluorescence in light

**$P < 0.01$; *$P < 0.05$
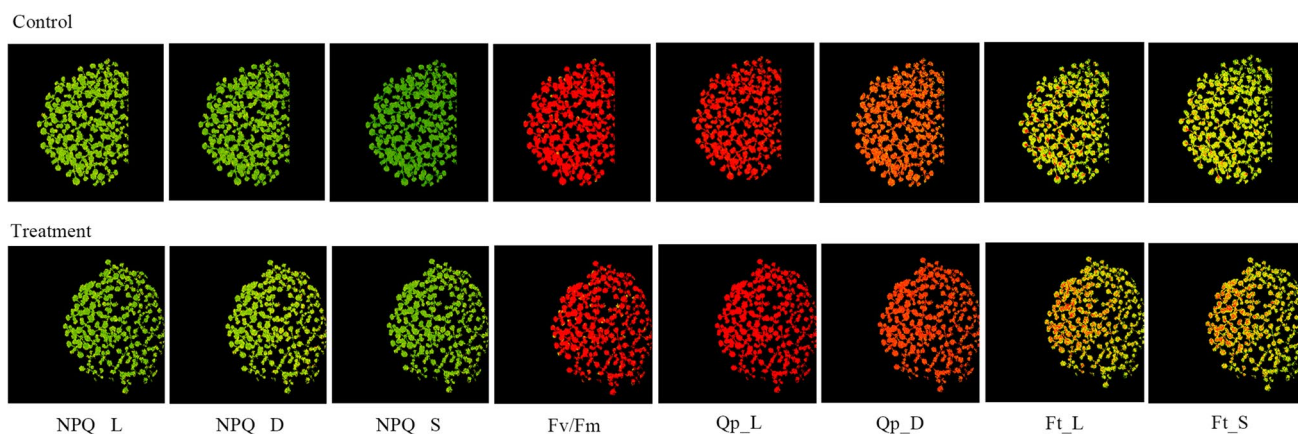
Fig. 7 Representative chlorophyll fluorescence imagery of *Arabidopsis thaliana* seedlings under control and 48-h microwave exposure conditions. The difference in nonphotochemical quenching (NPQ) and fluorescence (Ft) is visible in the images

tolerable level for seedlings. It is necessary to find the tolerable limit of H_2O_2 in a different study in order to confirm this phenomenon. When plants are under stress, lipid peroxidation of the cell membrane occurs due to elevated levels of reactive oxygen species, and MDA is produced (Weber et al. 2004). Thus, the reduced MDA content in the microwave-treated seedlings could prove to be a beneficial effect of microwaves on seedlings, though the H_2O_2 content is increased. The anthocyanin content was also reduced slightly in the microwave-exposed plants. When plants are under stress (drought, salinity, light, temperature, etc.), the anthocyanin content increases (Kovinich et al. 2015). The reduction in anthocyanin content recorded in the present study could be considered a beneficial effect of exposing seedlings to microwaves.

The NPQ is a mechanism that dissipates the excess excitation energy of photosystem II (PSII) as heat to protect the photosystem from excess light absorption, which leads to photoinhibition (Murata et al. 2012; Ruban 2016). In the present study, the increased NPQ in all stages (light phase, dark phase and steady state) of the treatment evidences that the photoprotection or thermal dissipation of the harvested energy was enhanced.

4.1 Unchanged F_v/F_m and increased NPQ and H_2O_2 –
The 0.81 F_v/F_m values of both the control and treatment seedlings reflect that under microwave exposure seedlings, maximum quantum efficiency was not affected; it is a sign microwave exposure does not affect the photosystem efficiency (F_v/F_m insensitive to microwave exposure), or adjustments of other fluorescence parameters prevented F_v/F_m values from being changed (Force et al. 2003; Kalaji et al. 2012). The increased NPQ of plants can be a result of the enhanced photoprotection of PSII and/or the increased absorption of light energy by the chlorophyll molecules, which is decapitated as heat. The light-harvesting complex of PSII mainly consists of Chl *b* (Kitajima and Hogan 2003; Kume et al. 2018). When the Chl *b* content is high in treatment samples, the PSII of those seedlings might absorb the excess light energy; however, to protect the photosystem from photoinhibition, the excess energy may have dissipated as NPQ and the reduced Ft is supporting the phenomena. On the other hand, NPQ can be increased in plants due to decreased CO_2 fixation activity or vice versa (Takács et al. 2014, Trimborn et al. 2014). However, the casually observed unchanged morphology, F_v/F_m, and QP suggested that the CO_2 fixations of treated seedlings were not affected by microwave exposure. In accordance with the present study, a previous study conducted a 48-h exposure experiment on *Myriophyllum aquaticum* (Vell.) Verdc. for horizontally polarized 2 GHz microwaves at 1.8 W m^{-2} and the H_2O_2 content increased significantly, but the morphology

was not affected (Senavirathna and Asaeda 2017). Therefore, the increased NPQ may not have resulted due to decreased CO_2 fixation; however, this should be confirmed with further research conducted for long-duration microwave exposure.

The Chl *a/b* ratio of the treatment was reduced compared to that of the control. A low Chl *a/b* ratio is an indication of increased light-harvesting proteins and thylakoid membranes (Anderson et al. 1995). On the other hand, a lowered Chl *a/b* ratio can be caused by oxidative stress damaged Chl *a* or by an increased Chl *b* content. In the present study, both the Chl *a* and Chl *b* contents increased in the treatment sample; the Chl *b* increased significantly than Chl *a*, which caused the lower Chl *a/b* ratio. As the excess excitation energy is dissipated via the NPQ, a portion of the excess energy can pass through the photosystem to produce excess H_2O_2 in tissues, which is evidenced in the present study. If photosynthesis is increased, it is expected that the biomass allocation would increase in the microwave-exposed seedlings. However, since the present experiment was conducted for 48 h only, this was not evaluated. Therefore, to evaluate the morphology and biomass allocation, extended research is required.

4.2 Further research direction –
The response of *A. thaliana* to microwaves is growth-stage dependent. When 5-week-old *A. thaliana* (Col) plants were exposed to the same microwave frequency and power density for the same duration (2.45 GHz, 1.0 ± 0.2 W m^{-2} at leaves, 48 h), they exhibited elevated H_2O_2 and Chl *a* and *b* contents; however, the POD activity was also elevated (Supplementary data 4). In addition, the germination rate of microwave-exposed seeds and the photosynthetic pigments of the seedlings of microwave-exposed seeds did not change. These results confirm the growth-stage-dependent response of plants.

Microwave properties, frequency, power density, polarization and exposure duration are determining factors of plant responses (Tkalec et al. 2007; Senavirathna et al. 2014; Senavirathna and Asaeda 2017). On the other hand, the growth stage and plant species are confirmed determining factors of the plant response to microwaves (Senavirathna and Asaeda 2014). Therefore, the response of plants to microwaves is complicated and must be discussed related to the plant characteristics as well as the microwave properties.

When light is considered, the frequency, intensity, polarization and exposure duration determine the plant response (Velez-Ramirez et al. 2011; Shibayev and Pergolizzi 2011; Kouřil et al. 2013; Bayat et al. 2018); the growth stage also affects the plant response to light. Therefore, in future studies, it is important to consider the light factor. Though the control and treatment plants experienced identical light conditions, the plants' light response might have been played a role in response to microwaves. This is especially plausible

because the most affected properties, the chlorophyll and H_2O_2 contents, are also affected by light intensity and light quality (Miller and Zalik 1965; Pospíšil 2016).

The results suggested that exposure to 2.45 GHz microwaves at 1.0 ± 0.1 W m^{-2} power density is advantageous for seedlings. The decreased Ft and increased NPQ suggested an increased acceptance of photon energy but released the excess energy as heat to protect the photosystem. Though the H_2O_2 levels of microwave-exposed seedlings were increased, the unchanged POD activity and decreased MDA content and unchanged F_v/F_m values suggested the nonexistence of oxidative stress in the seedlings, probably because H_2O_2 levels remained under the tolerance threshold. However, it is necessary to extend the research to study the possible morphological and physiological changes in seedlings that are continuously exposed for an extended duration of time.

Compliance with ethical standards

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