Effects of microwave doses on seed exposure

Abstract

A massive increase in electromagnetic pollution since the introduction of telecommunication instruments especially microwave from which the mobile communication. Current research study aim to assess the physiological effects of seed exposure to different doses of microwave. Microwave-induced electrolyte leakage, germination, chlorophyll and growth were monitored and evaluated following seed exposure to microwave from a magnetron of 2. 45 GHz, maximum output power of 800 W and wavelength of 12 cm operated at 220 VAC. seeds of Hordeum vulgare were exposed to eight different exposure periods of microwave from 0 to 600 seconds, experiments were performed *in vitro* . Percentage of germinated seeds, relative germination coefficient, germination rate, germination index, fresh and dry weights, shoot: root ratio were assessed. Germination parameters were dose-dependents, the percentage of germinated seeds were increased after short exposure periods to microwave recording 100 % germination. Further the germination rate, relative germination coefficient were also increased after short exposure periods to microwave. Longer exposure periods reduced the percentage of germination, germination rates and various germination indices. Morphological and growth traits showed a similar trend and were significantly decreased after longer exposure periods to microwave. Chlorophyll contents were significantly decreased with increasing exposure periods of microwave. Microwave-induced electrolyte leakage (%) was significantly increased (r= 0. 92*, p <0. 001) following pretreatment with different exposure periods of microwave. Current research data gives a spotlight on both possible positive and negative effects of microwave on barley and recommended to minimize the exposure time to environmental

microwave to safeguard recovering ability of biological systems. However, the positive effects of microwave are of considerable importance in improving crops.

Key words: Microwave, electromagnetic, barley, membrane ion leakage, cell death, germination, chlorophyll, SPAD, growth, *Hordeum vulgare* L.

Introduction

The Development of life was influenced by two ubiquitous forces; the gravity and electromagnetism, the two forces expected to have essential role in the functional activities of biological systems and organisms (Balmori, 2009). Previously, microwave radiofrequencies included a few radio and televisions transmitter located in remote area or high places. A massive increase in electromagnetic pollution since the introduction of telecommunication instruments in the 1990's (Galeev, 2000; Firstenberg, 2001; Ragha et al., 2011) (Ragha et al., 2011). These electromagnetic fields can have a deleterious and damaging effects depending on the exposure doses, power level, frequencies, pulsed or continuous wave and the dielectric properties of exposed tissue, the interaction of such electromagnetic fields on various life processes has been focused on different microwave frequency range forms an important part (Banik et al., 2003). Microwave are a part of electromagnetic radiations spectrum comprising frequencies ranging from 300 MHz to 300 GHz, further, it act through absorption on molecular level manifesting as vibrational energy or heat and a biological effects (Chipley, 1980; Dardanonl et al., 1985, 1994; Pakhomov et al., 1998)(Chipley, 1980; Dardanoni et al., 1985; 1994; Pakhomov et al., 2001) including various genetic changes. Relevant research suggests that microwaves may have long-term health effects (Lin, 2004). Identification, evaluation and assessment of the bio-effects of microwaves have been complex and controversial, because of the absence of a clear mechanism of the impact and interaction of microwave radiofrequencies and biological systems, there has been a persistent view in biophysical and engineering sciences, that microwave fields are incapable of inducing bio-effects other than by heating (Banik *et al.* , 2003). In recent times, non-thermal bio-effects of microwaves on tissue responses were being acknowledged (Dardalhon *et al.* , 1979a, b; Adey, 1981; Banik *et al.* , 2003).

Various research data have offered convincing evidence of non-thermal microwave effects and have also indicated various consistencies in these effects: dependency of frequency within specific frequency windows of " resonance-type"; dependency on modulation and polarization; dependency on intensity within specific intensity windows, including super-low power density comparable with intensities from base stations/masts (Adey, 1981; Belyaev, 2005; Hyland, 2000; Lai, 2005, (De Salles, 1999; Scialabba and Tamburello, 2002)). Some studies have demonstrated different microwave effects depending on wavelength in the range of mm, cm or m (Kemerov *et al.* , 1999; Nikolaevich *et al.* , 2001). Duration of irradiation may be as important as power density (Abu-Elsaoud, 2015), the effect of electromagnetic radiations could be depending on the radiation exposure dose representing a long-term cumulative influence (Adey, 1997; Galeev, 2000; Lai, 2005; Abu-Elsaoud, 2015). Modulated and pulsed radiofrequencies seem to be more effective in producing effects (Belyaev, 2005; Lai, 2005).

Low frequency modulations employ greater biological activity (Balmori, 2009).

Microwave irradiation could affect plant growth, development and seed germination (Hamada, 2007; Aladjadjiyan, 2010; Salama et al., 2011; (Scialabba and Tamburello, 2002; Monteiro et al., 2008; Ragha et al., 2011; RadzeviÄius et al., 2013; (Abu-Elsaoud, 2015). Low intensity microwave were reported not to affect the plant growth and development but the increased irradiation doses of microwave has decreased and slowed seed germination (Oprica, 2008). The direct effects of microwave on germination of cereals were studied by Ponomarev et al. (1996) where, a wavelength Î»= 1 cm and irradiation exposure dose of up to 40 minutes were applied to barley, oats, and wheat seeds leading to improved germination rate with optimum effect after 20 minutes of microwave exposure (Ponomarev et al., 1996). A study of irradiating vegetable seeds with high power microwave radiations reported a stimulation influence of various germination and growth rate parameters by microwave (RadzeviÄius et al., 2013). The effect of microwave irradiation with a different power on various seed germination consequences of four different ornamental crop species has been studied by Aladjadjiyan (2002). The electroconductivity of leaf extract were monitored and increase in various germination consequences were observed (Aladjadjiyan, 2002). A comparative effect of microwave radiations on germination and growth of six different Egyptian genotypes were assessed using different exposure times, his data supported a dose dependent possible stimulation effect of microwave on growth and germination (Abu-Elsaoud, 2015). The response of barley seedlings to microwave radiations of

2. 45 GHz after exposure to 0, 10, and 20 seconds of microwave radiations on four different genotypes (CreÈ›escu et al., 2013). Changes in peroxidase and catalase enzyme activities in *Brassica napus* were found to be dependent on microwave exposure time, seed condition and plant age (Oprica, 2008). The frequencies of the cell plasma membrane vibrations of bio-objects lie in the mm-wave range, that range is thought to be essential to any living organism. Microwave irradiations induce resonant phenomena within biological system and have a stimulatory effect on biological organisms (Aladjadjiyan, 2002; Yanenko et al., 2004). Most microwave irradiation studies focused on possible biological effects from phone masts and microwave radiofrequencies on animal and human health (Santini *et al.* , 2003; Hutter *et al.* , 2006; Balmori, 2009). The biochemical mechanism by which microwave radiations affect biological systems of living organisms is not fully comprehended and the mechanism could vary according to the amplitude, frequency and the irradiation duty cycle (Monteiro *et al.,* 2008; Aladjadjiyan, 2010). The present study was conducted to study the effect of seed irradiation with different doses of microwave radiations on the membrane electrolyte leakage, germination and growth of Egyptian barley *Hordeum vulgare* L seedlings.

Materials and methods

Plant materials

Seeds of selected Barely *Hordeum vulgare* L. genotype Giza-129 were acquired from Agricultural Research Station at Ismailia, Agricultural Research Centre (ARC), Giza, Egypt in the months of November-December, 2016. The cereal lot of seeds was cleaned removing unwanted matter and damaged seeds.

Radiofrequency irradiation treatment

Microwave radiofrequency irradiation were carried out using a magnetron with frequency of 2. 45 GHz, wavelength of 12 cm, a maximum output power of 800 W, maximum intensity were estimated to be 51. 5 kW. m $^{-3}$ by dividing the output power to the working volume m $^{-3}$. Experimental details were presented in diagram (1). Seeds were first soaked in distilled water for 1 hour recommended by Aladjadjian and Svetleva (1997) to enhance the absorption of microwave energy. Seeds of selected barley genotype *Hordeum vulgare* cv. Giza-129 were divided into eight groups, each variant containing 30 seeds of (three replicas of ten seeds). The first group represent the untreated control and remaining seven variants were irradiated with different exposure periods to microwave (1, 5, 10, 30, 60, 300 and 600 seconds).

Various germination traits were estimated and monitored during the experiment at different time-points; 3, 5, 7, 9 and 12 days after sowing (DAS). Based on the obtained results, the percentage of germinated seeds N_k , germination rate S_k (seed. h $^{-1}$), maximum number of germinated seeds, relative germination coefficient (W_k) were calculated with the using germination formulas by Ciupak *et al.* (2007) presented in Table (1).

Biomass and biomass allocation

Shoot and root biomass were determined for Triticum aestivum plants irradiated with 2. 45 GHz radiofrequency and the untreated control. Biomass allocation within plants was calculated in g per g (S/R ratio, g. g^{-1}) of total seedling biomass to avoid size effects, and calculated as a mean of three replicas. Data of Biomass allocation and shoot-to-root ratios were assessed statistically in plants irradiated with microwave radiofrequency versus the control ones to evaluate the change in biomass allocation pattern.

Statistical analyses

Analysis of variance test (ANOVA) followed by Duncan's multiple range comparisons were employed to analyse the results of barley after seed irradiation with microwave radiations. Further, correlation and simple linear regression analyses were also performed using SPSS statistical software ver. 22 and Microsoft Excel package 2016 at a confidence level of 95%.

Results

Seed germination

The influence of microwave radiations on various germination dynamics were assessed intensively on the first twelve days after seeds sowings (DAS) in *Hordeum vulgare* L. plant. Barley seeds were subjected to different exposure doses of microwave radiation from magnetron with 2450 MHz and 800 Watts. Germination indices monitored and assessed are; number of germinated seeds (n_k), percentage of germinated seeds (%), germination rate (Sk; seed. h^{-1}), germination index (GI), and the relative germination coefficient (W_k) at different time points 3, 5, 7, 9, and 12 DAS (days after seeds sowing). The percentages of germinated seeds were presented in Figure (1A-E) for different time points. A significant change in the percentage of germinated seeds were observed after seed irradiation with microwave assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range comparisons. Significant variations were observed versus the untreated control plant group. Letters on figures 1 (A) to (E) represent the results of Duncan's multiple range comparisons, where, different letter mean significant difference (Figure 1). The maximum germination percentage observed were 100% recorded at MW dose of 5 seconds-5 DAS, 1, 5 seconds dose 7, 9, 12 DAS. MW radiations observed to have a positive effect on germination at low doses of 1, and 5 seconds (Figure 1) these were assessed statistically by ANOVA and Duncan's multiple comparisons.

The general trend of MW radiations on seeds germination percentage was strong negative and significant relationship (Figure 3A-E) revealed by both regression and Spearman's correlation i. e. increasing levels of MW radiations caused decrease in germination parameters especially high doses of MW.

Other germination indices e. g. germination rate (S_k; seed. h^{-1}) were also recorded at different MW doses and time points (3, 5, 7, 9, 12). Germination rate in the untreated control 0. 19 seed. h^{-1} 5 and 7 days after seed sowing while in seeds treated with 1 and 5 seconds of MW the germination rate increased from 0. 19 to 0. 21 seed. h^{-1} revealing that not only the germination percentage increased but also the germination rate and speed (Figure 2A-D). Further, early germination was recorded after 1 and 5. Figures 3 (F-I) represent linear regression trend-line for the effect of MW radiation on germination rate, which had a strong inverse significant effect.

Relative germination coefficient (W_k) were calculated and normalized to the control germination. Data of relative germination coefficient were presented in Figures (2E-H) at different time points (3, 5, 7, 9); respectively. The relative germination coefficient increased after MW irradiation of 1 and 5 seconds (Figure 2E-H), while, W_K was decreased after irradiation with higher doses of MW radiations. Analysis of variance was carried out to assess the different between treatments control and were followed by Duncan's multiple range comparisons. Linear regression trend-lines presented in figures (3K-N) represent the linear relationship between MW radiation doses and relative germination coefficient (W_K) after 3, 5, 7, 9 days after seed sowing. Inverse strong significant relationship between increasing doses of MW radiations and W_K. The germination index (GI) followed the same trend with increasing levels of microwave radiations (Figures 1, 3O).

Photosynthetic pigments and Growth:

Shoot and root biomasses were estimated in *Hordeum vulgare* L. plants after irradiation to MW radiations. Shoot biomass ranged from 0. 03 to 0. 42 g/plant-FW where the maximum shoot fresh weight recorded after MW irradiation of 1 second dose and minimum in 600 seconds. MW radiations severely decreased the shoot biomass in barley (Figure, 4A). Root biomass, on the other hand, ranged from 0. 03 to 36 g/plant-FW. The highest root fresh weight was recorded at 300 s MW irradiation dose. While minimum root fresh weight were recorded after 600 seconds MW dose (Figure 4B). Whole plant fresh weight ranged from 0. 06 to 0. 67 g/plant-FW. Shoot, root, and whole plant biomass showed a negative trend with increasing levels of MW radiations revealed by simple linear regression analysis and Spearman's correlation (Figure 7A, B, C). Microwave irradiation induced a significant decrease in shoot, root, and plant biomass in barley plants (Figure 7A, B, C).

The behaviour or nutrient allocation was assessed in terms of shoot and root biomass as shoot: root ration (g. g^{-1}) after seed irradiation with MW. Biomass behaviour was allocated toward barely shoot system after irradiation with 1 seconds of MW radiations. While, higher doses of MW induced nutrients to be allocated toward root system (figure 5B, 3O).

Leaf chlorophyll contents increased significantly after 1 and 5 seconds of MW irradiations compared to the control (Figure 5A), however, MW doses from 300 and 600 seconds decreased significantly from the untreated control. Plant height was monitored after various MW irradiations doses and showed a significant decrease in response to MW (Figure 5) revealed by Duncan's multiple range comparisons versus untreated control plants. Root volumes did not changes significantly with MW radiations except for the 600 seconds dose which showed a significant decrease versus control (Figure 5D)

Membrane Ion leakage (%)

Electrolyte leakage is a stress-induced injury that commonly used as a measure of plant response and tolerance to stress (Bajji et al., 2002; Lee and Zhu, 2010). MW irradiation with dose 1 and 60 seconds did not induce a change in electrolyte leakage; however, MW doses 5, 10, 30, 300 and 600 seconds significantly increased the electrolyte leakage compared to the untreated control (Figure 6). A strong negative significant relationship between increasing doses of MW radiations and electrolyte ion leakage ($R^2 = 0.84$; Pearson Correlation= -0. 61; p -value <0. 002*).

Discussion

Microwave irradiation with different exposure doses induced changes in various parameters of barley (*H. vulgare* genotype Giza-129). Germination parameters were dose-dependent and were stimulated by several exposure doses of microwave radiations. The percentage of germinated seeds, germination rate, relative germination coefficient and germination index at different time points were increased by short exposure to microwave radiations, however, height exposure doses of microwave-induced a significant decrease in germination consequences. Further, various growth parameters were increased by one or more low doses of microwave radiations and were significantly decreased by higher exposure doses. These results were found to be in agreement with (Abu-Elsaoud, 2015; Aladjadjiyan, 2002; CreÈ›escu et al., 2013; Ragha et al., 2011). Seed germination is completed with the protrusion of the radicle through the seed coat (Bewley & Black, 1994). The subsequent seedling growth involves the establishment of the root and shoot systems. The hypocotyl growth is caused principally by cell expansion and/or by elongation. The low power 10. 5 GHz irradiation reduces the rate and percentage of germination in radish seeds and increases germination mean time, thus impairing seed germination. The germination reduction is linearly dependent on the MW power intensity incident on the seed. These findings support the simplified hypothesis that the power density on a plane perpendicular to wave direction decreases with the inverse square of the distance from the source.

Membrane electrolyte leakage accompanies the plant response to stresses were monitored at different microwave exposure doses. Electrolyte leakage is widely used as a measure of stress-induced injury in plants (Bajji et al., 2002; Lee and Zhu, 2010). According to our results microwave radiation with dose 1 seconds and 60 seconds did not induce a change in electrolyte leakage; however, MW doses from 5 to 600 seconds significantly increased membrane electrolyte leakage compared to the untreated control. These results in agreement with previous results (Aladjadjiyan, 2002; Demidchik et al., 2014). A possible explanation by (Aladjadjiyan, 2002) suggests a hypothesis about the absorption of the microwave radiation energy by the hydrogen or magnesium atom's electrons in the chlorophyll molecule. The energy absorbed is redistributed and it causes changes in the chlorophyll molecule. By increasing the radiation power used for the treatment of the samples, the number of free ions in the extract decreases and hence its electroconductivity, too (Aladjadjiyan, 2002). Studies using patch-clamp method showed that the microwave exposure reduces trans-membrane protein channels opening in cultured chick myotubes probably because microwaves provoked an alteration of intracellular enzymatic processes e. g. protein kinase activation (D'Inzeo et al., 1988) (D'Inzeo *et al.,* 1988). In plant cells, the protein of water channels namely aquaporins of vacuolar membranes and plasma membranes are involved in the regulation of water movement dynamics in growth and development of plant cell and in stress responses (Maurel, 1997). In case of radish seedlings, microwaves may reduced water passage across cell membrane blocking aquaporins and causing reduction of growth in a turgor-dependent manner (Scialabba and Tamburello, 2002).

The increase of growth rate upon irradiation removal shows that during the elongation growth, the cell can partially repair damages occurred at the membrane level. There is a general consensus of opinion about the fact that MW induces a thermal detrimental effect over the biological system. In the present case, we assume that the damage induced by the low- power microwave exposure is non-thermal because a slight temperature increase (up to 25 ^{o} C} over radish seeds has been demonstrated to induce germination and growth increase (Scialabba & Melati, 1995). The reduced germination percentage and the delayed seedling growth confirm the importance of a serious cause of concern about the influence of expo- sure to environmental MW fields. It can be stressed the importance of limiting in time the exposure to MW as suggested by the recovering ability of the biological system considered in the present research.

Membrane Electrolyte leakage is an essential measure of the plants' responses to various stresses. It is mostly associated with the K^+ efflux, which is a common response in plant cells (Demidchik et al., 2014). The stress-induced electrolyte leakage is always accompanied by reactive oxygen species (ROS) generation and hence, leads to programmed cell death. Recent results exhibited that reactive oxygen species (ROS; H_2O_2 and hydroxyl radicals) activates annexins, SKOR and GORK genes that catalyses K^+ efflux from plant cells (Demidchik et al., 2014). Further, GORK-genes mediated potassium ion (K^+) cause programmed cell death under oxidative stress. The intracellular endonucleases and proteases look to be blocked by potassium ions; consequently, the efflux of these K^+ stimulates these nucleases and proteases hydrolytic enzymes causing programmed cell death (PCD). Potassium ions could play a “ metabolic switch” role under moderate stress conditions decreasing the anabolic reactions rate and stimulating catabolic reactions, leading to the release of energy required for repairing and adaptation needs (Demidchik et al., 2014).

The effect of microwaves on plants was the main purpose of the current study. Since it is a known problem, many other pieces of research were done on this topic. Having seen and observed other projects, we noticed that the major conflict was between whether microwaves affect plants germination or not. Our hypothesis was that they do affect it and, of course, it is well known that they do but it still made a challenge trying to prove it and it was found that every single step affected the results. Since it is likely that other people who did similar projects have done some errors through their study, the results were not reliable and could not be considered accurate enough.