

Microwave radiation, seed germination and seedling growth responses in pepper (*Capsicum annuum* L.)

Abstract

The propagation of pepper (*Capsicum annuum* L.) generally occurs through seed. Seed germination is a critical stage in the life-cycle of the plant, and often controls plant population dynamics. Here, we report an effective duration of exposure to microwave radiation to improve seed germination and growth indices of *C. annuum*. Two experiments were carried out at the seed germination and seedling growth stages. The results revealed that exposing the seed to 2,450 MHz microwave frequency radiation for ten hours resulted in the highest seed germination percentages (SGP). This treatment led to a SGP of 64.7% after 14 day (SGP₁₄), with a mean germination time (MGT₁₄) of 9.5 d. Although, other duration of exposure to microwave radiation had moderate effects on SGP₁₄ from 42 to 64%, they failed to reduce MGT₁₄ values. Treating pepper seedlings with microwave frequency radiation revealed that 1 h of exposure increased plant growth indices (such as plant height and numbers of leaves), while longer exposure times (3 h or 4 h), limited growth. Interestingly, longer durations of exposure to microwave frequency radiation changed the behaviour leaves. This technique may be therefore used to enhance seed germination percentages and seedling growth in pepper.

Keywords: *Capsicum annuum*, chlorophyll content, germination rate, microwave frequency, microwave radiation

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Introduction

Applications of microwave energy in agriculture have increased over the past 50 years.¹ The range of microwave frequency is between 300 MHz - 300 GHz.^{2,3} Due to their high frequency and short wavelength, microwaves are desirable for communication and radar applications. Microwaves are an interesting research subject, even in the 21st century. Some research has been done on the effects of microwave on seed germination in species such as wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum*), green gram (*Vigna radiate*), and moth bean (*Vigna aconitifolia*), under microwave conditions. It has been reported that a threshold level of microwave energy and exposure time can improve seed germination and seedling vigour in plant species,⁴ while further increases in microwave frequency, energy or density caused a reduction in SGP and seedling vigour.⁴ Pepper (*Capsicum annuum* L.) is one of the most popular vegetables grown throughout the World, especially in South-East Asian countries such as Bangladesh, India, Pakistan, and Sri Lanka. Peppers are rich in vitamin C, vitamin A, and calcium compounds with anti-oxidant and anti-carcinogenic activities.⁵ Mature fruit also contain high concentrations of anti-oxidant phenolic compounds.⁵

Microwave energy has been important for electrochemical-based investigations on biological behavior of seed germination⁶ and enzyme activities⁷ in different plant species. The objective of the present study was to investigate the effects of microwave frequency radiation on seed in *C. annuum* seed by measuring SGP values and GR, as well as measuring various morphological and physiological traits.

Materials and methods

Seeds of *Capsicum annuum* L. were provided L. by the Agro Gene Bank of the University Putra Malaysia, Serdang, Malaysia.

Microwave equipment

The microwave antenna used was a 2.4 GHz dipole antenna, 108.5 mm in length (GW.15.2113 model; USA) with a frequency controlled oscillator (ZX95-2490 model; Mini-Circuits; USA) including linear tuning characteristics such as a frequency range from 2,280–2,490 MHz, low phase noise, and low pushing, low pulling.² The amplifier used (Model ZX60-3011; Mini-Circuits; USA) was a 50 Ω, 400–3,000 MHz low-noise model. The other features were a high dynamic range, a wide band-width, a low noise (1.5 dB) feature, 1dB compression, and medium IP3 (www.minicircuits.com). The detectors used (Models ZX47-50 and ZX47-50LN; Mini-Circuits; USA) were 50 Ω, from -50 dB m to +15 dB m, and 10-8,000 MHz co-axial power detector's with a low-noise DC output, 20 mVp-p type at 10 MHz, a high dynamic range, and a high band-width.

Experimental design

Two experiments were carried out at the seed germination and seedling growth stages from July - September 2012. The first experiment was based on a randomised complete block design (RCBD) with five treatments (five different durations of exposure to microwave frequency radiation at 2,450 MHz), each with three replicates. The five treatments were C (control), T1 (1 h), T2 (4 h), T3 (7 h), and T4 (10 h). All seeds were surface sterilised in 10% (v/v) sodium hypochlorite for 10 min,⁸ then rinsed thoroughly with distilled water. Fifty seed were then soaked in each of 15 Petri dishes (five treatments×three replicates) containing filter paper moistened with sterile water. The Petri dishes were incubated in a controlled growth chamber (6MP6010; Adaptis, Conviron) at 28–30°C and a relative humidity of 70–85%. The Petri dishes were then given five different durations of exposures to microwave radiation in a 60 cm×60 cm×60 cm dimensions box covered in aluminium foil. Seed germination was recorded every day 8 day after exposure. Seed germination after 8 day

(SGP₈) and 14 day (SGP₁₄) were calculated. The germination rate on day-14 (GR₁₄) was calculated by dividing the SGP obtained from each count by the number of days. The mean germination time (MGT₁₄) was calculated using the formula of Ellis & Roberts⁹

$$MGT = \sum D_n / \sum n$$

Where MGT was the mean germination time, n was the number of seeds which germinated on day D, and D was the number of each day counted from the start of germination. The design of second the experiment was the same as the first experiment. Seedlings were grown in the mixture of top soil, peat moss and sand (2:1:1 v/v) in 15 cm diameter pots. All plants at 10-12 leaf stage were exposed to different durations of microwave frequency irradiation, with three replicates. During the irradiation process, plants were incubated in a growth chamber at 28-30°C, at a relative humidity of 70-85%, with a 12 h photoperiod. All experiments used 2,450 MHz microwave radiation at 1.58 mW. The plants were placed 15 cm from the antenna and the durations of exposure were 1, 2, 3 and 4 h, repeated each day for 20 day. Space restrictions due to the dimensions of the box meant that only six pots (one seedling per pot) could be treated. The pots were located 15 cm from omni-directional microwave antenna used for irradiation. Due to the circular pattern of irradiation, the pots surrounded the antenna (Figure 1). Treatments T1 and T2 were first. After 1 h, the T1 samples were removed and replaced with the T4 pots. After 4 h from the start, the T2 samples were removed and the T3 samples were placed in the box, and finally after 7 h and 10 h the T3 and T4 samples were removed, respectively. The numbers of leaves (NL), plant heights (PH), mean leaf lengths (MLL), mean leaf width (MLW) and chlorophyll content were measured 10 day and 20 day after the end of the irradiation treatments.

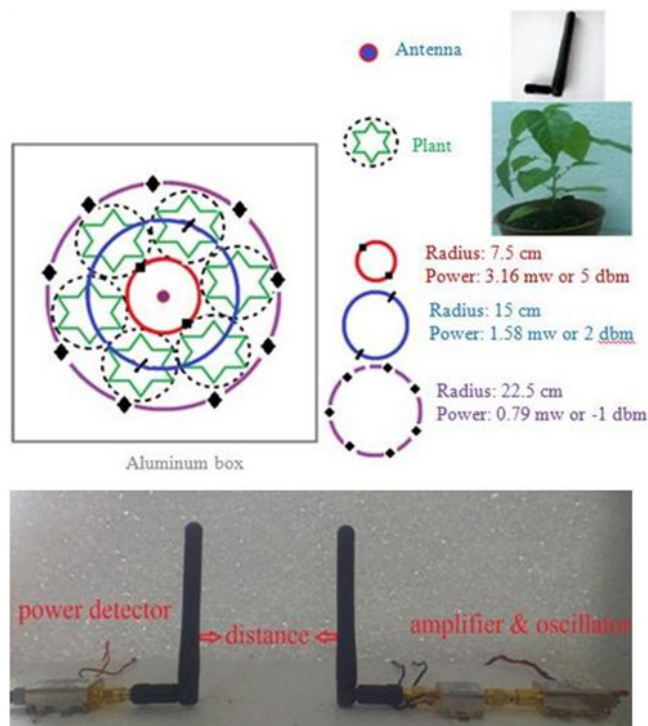


Figure 1 Location of pots and antenna inside the box.

Determination of chlorophyll content

Chlorophyll contents in leaves were measured using a chlorophyll-meter (XT-SPAD-502). The absorbance of chlorophyll was measured at 650 nm and non-specific absorbance was recorded at 940 nm. Since the area measured by the SPAD-502 was only 6 mm², a minimum of six reading were taken per leaf, then the average value was recorded.

Statistical analysis

Data were tested for normality using SAS Version 9.¹⁰ The data for the traits studied were analysed using analysis of variance and Duncan's multiple range test at significance level of $P \leq 0.01$.

Results

Effect of different durations of exposure to microwave frequency radiation on seed germination in *Capsicum annuum*

Different durations of exposure to microwave frequency radiation prior to seed germination significantly accelerated the rate of germination of *C. annuum* seed. Analysis of variance of the treatment effects on SGP₈, TSGP₁₄, MGT₁₄, GR₁₄, RL, and SL showed that the treatments significantly affected the SGP of seed (Table 1). Seeds germinated successfully 8 day after T1 – T4 with SGP₈ 6 - 20%. The SGP₈ values showed that 10 h of microwave frequency radiation was the effective time to germinate the seed in which 20% of the seeds germinated after eight days (Figure 2a). This SGP value increased to 64.7% after 14 day (Figure 2b). Other durations of exposure to microwave frequency radiation showed varying effects as reflected by SGP₈ values ranging from 6-14%. Exposing the seeds for short times (4h) with microwave frequency improved the GR as shown by the GR₁₄ value (Figure 2c), but increasing the exposure time led to decrease in GR. The highest SL and RL were obtained at 4 h exposure to microwave frequency, while exposing the seeds for long time decreased the SL and RL (Figure 2d).

Table 1 Analysis of variance of the effect of different duration of exposure to microwave frequency radiation on germination traits in *Capsicum annuum* seed

S.O.V	df	Mean squares					
		SGP ₈	TSGP ₁₄	MGT ₁₄	GR ₁₄	SL ₁₄	RL ₁₄
R	2	12.8 ^{ns}	17.87 ^{ns}	0.07 ^{**}	0.13 ^{ns}	0.42 ^{ns}	0.25 ^{ns}
T	4	90.00 ^{**}	264.40 ^{**}	0.45 ^{**}	1.83 ^{**}	2.51 ^{ns}	7.40 ^{**}
Error	8	6.80	7.20	0.01	0.10	2.91	0.26

** and ns, refer to $P \leq 0.01$ and non-significant, respectively. S.O.V: source of variance, df: degree of freedom, R: block, T: treatment, SGP₈: seed germination percentage after 8 days, TSGP₁₄: total seed germination percentage after 14 day, MGT₁₄: mean germination time after 14 days, GR₁₄: germination rate after 14 days, SL: shoot length, RL: root length.

Effect of microwave frequency on the growth of *Capsicum annuum*

Microwave frequency affected the plant height (PH) of *C. annuum* seedlings after 10 d exposure and PH and NL after 20 d exposure. Variation due to microwave frequency on PH and NL was highly significant ($P \leq 0.01$), while there were no significant differences on

other measured traits after 10 day and 20 day exposure (Table 2). All studied morphological traits increased under the effect of microwave frequency. Among the different durations of exposure to microwave frequency radiation, 1 h gave the highest PH, NL, MLL and MLW after 10 day and 20 day, followed by 4, 3 and 2 h exposures. Increasing the exposure duration to microwave frequency radiation increased the

values of studied morphological traits (Table 3). Mean of PH and NL after 20 day varied significantly between 21.33 cm (control) to 25.67 cm (1 h exposure) values and 14 (control) to 18.33 (1 h exposure), respectively (Table 3). Overall, 1 h exposure to microwave frequency radiation was best with respect to the morphological traits studied.

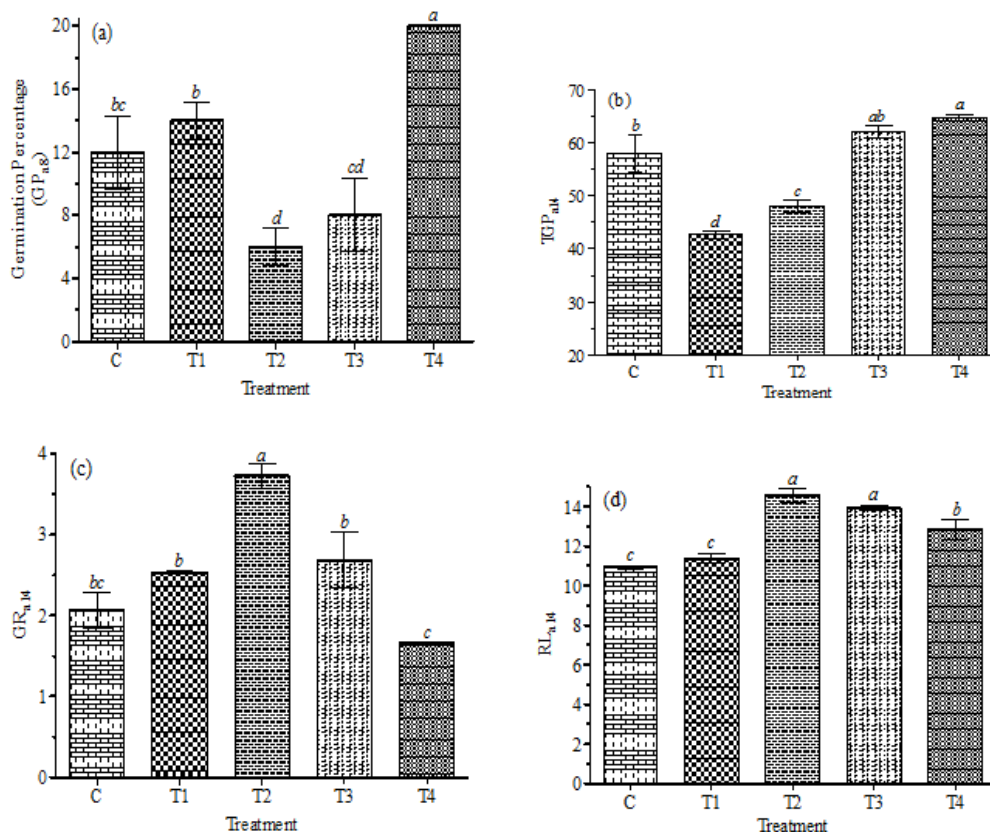


Figure 2 Seed germination in *Capsicum annuum* following different duration of exposure to microwave frequency. Germination percentage 8 day after treatment (a), Germination percentage 14 days after treatment (b), Germination rate 14 days after treatment (c), and Root length of germinated seeds (d). Mean values±S.E are from three independent replicates. Mean values in columns superscripted by different letters are significantly different by Duncan’s multiple range test (P≤0.01).The treatments were: [C] Control, [T1] 1 h [T2] 4 h, [T3] 7 h and [T4] 10 h exposure to2450 MHz microwave frequency duration.

Table 2 Analysis of variance of the effects of the different duration of microwave on selected morphological traits in *Capsicum annuum*

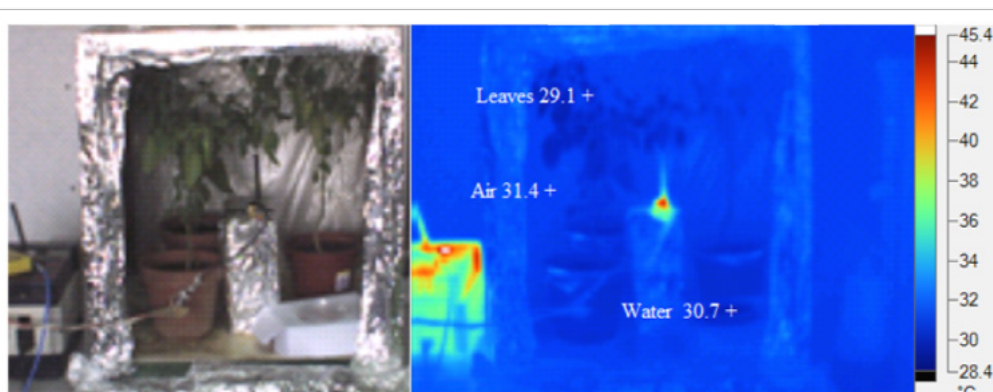
S.O.V	df	After 10 days					After 20 days				
		NL	PH	MLL	MLW	CHLO	NL	PH	MLL	MLW	CHLO
R	2	2.40 ^{ns}	1.77 ^{ns}	1.24 ^{ns}	0.19 ^{ns}	3.71 ^{ns}	3.27 ^{ns}	1.91 ^{ns}	4.40 ^{ns}	0.65 ^{ns}	5.15 ^{ns}
T	4	4.67 ^{ns}	8.95 ^{**}	3.54 ^{ns}	1.23 ^{ns}	8.01 ^{ns}	11.17 ^{**}	7.38 [*]	4.47 ^{ns}	1.08 ^{ns}	6.73 ^{ns}
Error	8	1.57	1.47	1.37	0.49	10.98	1.77	1.49	1.49	0.35	13.40

** , * and ns, refer to P≤0.01, 0.05 and non-significant, respectively. S.O.V: source of variance, df: degree of freedom, R: block, T: treatment, NL: number of leave, PH: plant height (cm), MLL: mean leaf length (cm), MLW: mean leaf width (cm), CHLO: chlorophyll (µg.g⁻¹ FW).

Table 3 Comparison of different durations of exposure to microwave frequency radiation based on morphological traits in *Capsicum annuum*

Treatment	NL ₂₀	PH ₂₀	MLL ₂₀	MLW ₂₀	CHLO ₂₀
Control	14.0±0.0 ^b	21.3±0.3 ^b	9.0±0.2 ^b	4.6±0.2 ^b	35.8±0.0 ^a
T1	18.3±0.3 ^a	25.7±0.2 ^a	11.8±0.2 ^a	6.0±0.2 ^a	36.3±1.0 ^a
T2	13.3±0.7 ^b	22.7±0.5 ^b	8.8±1.0 ^b	4.4±0.4 ^b	35.9±3.4 ^a
T3	15.7±1.7 ^b	23.0±1.3 ^b	9.2±1.4 ^b	5.0±0.6 ^b	35.7±0.4 ^a
T4	15.3±0.3 ^b	23.3±0.7 ^b	10.1±0.5 ^{ab}	4.9±0.3 ^{ab}	39.2±2.7 ^a

Data are mean values of three independent measurements ± standard errors of mean. The different letters indicate significant differences based on Duncan's multiple comparison test at $P \leq 0.01$. NL: number of leaves, PH: plant height (cm), MLL: mean leaf length (cm), MLW: mean leaf width (cm) and CHLO: chlorophyll ($\mu\text{g}\cdot\text{g}^{-1}\text{FW}$).

**Figure 3** Thermograph examination after 4 h exposure to 2450 MHz microwave frequency duration inside the box.

Discussion

Plants, especially vegetables, play an important role in our life and this issue has become urgent over the years. In this regard, germination is a critical stage in the life cycle of weeds, medicinal plants and crop plants, and often controls population dynamics, with major practical implications.¹⁰ The propagation of *C. annuum* L. generally occurs through seeds and germination of seeds depends on seed structures and the environmental factors such as temperature that affect the growth potential of the embryo.¹² In order to determine the germination and growth index of *Capsicum*, germination and growth parameters like SGP, MGT, GR, PH, NL, MLL, MLW and chlorophyll content were evaluated under different durations of exposure to microwave frequency radiation. All the studied germination indices and growth parameters were affected by different durations of exposure to microwave frequency radiation. Thermograph examination revealed that increasing the exposure to microwave frequency led to increase in the temperature of the water inside the Petri dishes around the seeds. The temperature of the water inside the Petri dishes after 1 h and 10 h exposure to microwave frequency were 29 to 34.1 °C, respectively. Therefore it can enhance the movements of molecules and improve the growth potential of the embryo. Our results matched up well with the findings of Nelson,² Tran¹³ and Vadivambal,¹ who showed that microwave frequency radiation, facilitated the GR and growth indices of *C. annuum*. This may be due to the positive effects of microwave frequency on water absorption and other biochemical processes.² The temperature of the environmental conditions inside the box after 1h and

4 h exposure to microwave frequency were 29-31.4 °C, respectively, and the temperature of the plant (leaves) inside the box after one and four hours were 28.2-29.1 °C (Figure 3). Increasing durations of exposure to microwave frequency radiation led to linear increase in temperature of environment around the plant inside the box, while the temperature of plant leaves was non-linear. In the initial period (after 1 h), the plants were in the process of adapting to microwave frequency due to increase in temperature, but in the next periods, the plants were able to withstand the temperature due to the release energy, which may be led to limitation in plant growth. Under short exposure to microwave frequency (1 h), the energy facilitated the plant growth as indicated by the increase in certain measured morphological traits such as PH and NL in the plant similar to the results of Ferriss,¹⁴ while under long exposure time of microwave frequency (3 h and 4 h) the growth was limited. Interestingly, long exposure to microwave frequency caused changes in performance of leave, which may lead to decline in photosynthesis capacity, respiration, and disturb metabolism of several cellular components such as protein synthesis. According to Basra,¹⁵ and Baskin & Baskin¹⁶ reports, germination stage needs higher temperature than growth stage in the plants. Because of this long exposure duration led to increase in the SGP and GR, while short exposure led to improve on growth indices. Our results revealed the important role of microwave frequency in improving the seed SGP, GR and plant growth. The overall results suggested that 10 h and 1 h exposure to microwave frequency as the best exposure to microwave frequency for germination and growth of *C. annuum*, respectively.¹⁷

Conclusion

The most impact of microwave radiation on the seed germination had the greatest effectors after 10 h exposure and on seedlings growth after 1 h exposure. The mechanisms of microwave frequency on seed germination and seedling growth are unknown. But according to positive effects of the microwave frequency on SGP GR and plant growth it can be concluded that the microwave energy can increase the temperature surrounding the seeds and plants and facilitate the SGP, GR and plant growth. Interestingly, long exposure to microwave frequency caused changes in performance of leaves, which can be applied to produce mutant plants in plant breeding programme.

Acknowledgments

None.

Conflicts of interest

Author declares that there is no conflict of interest.

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