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The effect of strong microwave electric field radiation on: (1) vegetable seed germination and seedling growth rate

Audrius RADZEVIČIUS¹, Sandra SAKALAUŠKIENĖ¹, Mindaugas DAGYS²,
Rimantas SIMNIŠKIS², Rasa KARKLELIENĖ¹, Česlovas BOBINAS¹, Pavelas DUCHOVSKIS¹

¹Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry
Kauno 30, Babtai, Kaunas distr., Lithuania
E-mail: a.radzevicius@lsdi.lt

²Center for Physical Sciences and Technology
Go tauto 11, Vilnius, Lithuania
E-mail: dagys@pfi.lt

Abstract

The effect of high power microwave (HPM) irradiation on seed germination and seedlings was evaluated. Vegetable seeds were subjected to HPM irradiation. The main focus was on the thermal heating elimination during seeds irradiation. For that reason short high frequency microwave pulses were used. The study object was seeds of different harvest years (2000, 2003 and 2008) of radish (*Raphanus sativus* L.) variety 'Babtų žara', tomato (*Lycopersicon esculentum* Mill.) variety 'Viltis', carrot (*Daucus sativus* Rohl.) variety 'Vaiguva' and tomato (*Lycopersicon esculentum* Mill.) variety 'Red Cherry' (seeds harvested in 1980). In the first experiment, all seeds were exposed to microwaves at 9.3 GHz frequency for 10 min. In the second experiment, carrots 'Vaiguva' seeds (11 years-old) were exposed to microwaves at 9.3 GHz frequency for 5 and 20 min and exposed to 2.6 and 5.7 GHz microwaves for 10 min. To establish microwave effect on seedling growth rate, tomato, carrot and radish seeds harvested in 2008 were exposed to microwaves at 9.3 GHz frequency for 10 min. It was established that 11 year-old radish seeds exposed to 9.3 GHz microwaves had higher germination as compared with non-irradiated seeds. HPM increased radish germination energy by 6% in seeds harvested in 2003. HPM exposure significantly increased the germination energy and germination of 8 year-old carrot seeds. The highest carrot seed germination was established at 9.3 GHz microwave frequency under 5 min exposure. Seed exposure to HPM (9.3 GHz) had a significant positive effect on dry weight of tomato seedling shoots and on tomato and radish seedling height, but it had negative influence on carrot seedling height. Seed irradiation with HPM (9.3 GHz) had a significant positive effect on the amount of chlorophyll *a*, chlorophyll *b*, chlorophylls *a + b* and carotenoids in tomato seedlings' fresh mass, but the opposite effect was determined in carrot seedlings' fresh mass.

Key words: *Daucus sativus*, irradiation, *Lycopersicon esculentum*, photosynthetic pigments, *Raphanus sativus*.

Introduction

Microwaves are part of the electromagnetic spectrum and are considered to be that radiation ranging in frequency from 300 million cycles per second (300 MHz) to 300 billion cycles per second (300 GHz), which correspond to a wavelength range of 1 m down to 1 mm. This nonionising electromagnetic radiation is absorbed at molecular level and manifests as changes in vibrational energy of the molecules or heat (Banik et al., 2003).

A wide-ranging review of the effects of various forms of externally applied electrical energy upon plants and organisms is presented. Most of the papers describe microwave experiments using 12.25 cm wavelength which corresponds to a frequency of 2450 MHz. This is one of the frequencies allocated, by international agreement, to microwave power devices for domestic and industrial applications. So we can find information about thermal influence on biological objects using microwave ovens. Also, it is very important to investigate the influence of high power microwave (HPM) pulses on the plants and

their seeds trying to distinguish between heating and direct influence of strong electric field on them. There is very little information about the effect of HPM pulses on the various agricultural crops.

The existence of specific (non-thermal) biological effects of extremely high power microwave pulses still remains discussable. The fact that extremely high power microwave can certainly produce a thermal effect makes it technically difficult to discriminate its possible specific effect in experiments (Pachomov, 1998; Tylkowska et al., 2010). Scientists made calculations and came to the conclusion that the thermal effect of short microwave pulses, when hygienic limits are not exceeded, is not able to make any chemical or structural changes in the biological tissue, even if a worst assumption of 100% absorption of a maximum (allowed by International Commission for Non-Ionizing Radiation Protection) pulsed energy in a thin protein monomolecular layer within an extremely short time is considered. In addition,

there is no physical cause for expectation of another effect of pulsed high frequency fields than the thermal effect (Šistek, 2002; Racuciu, Miclaus, 2007).

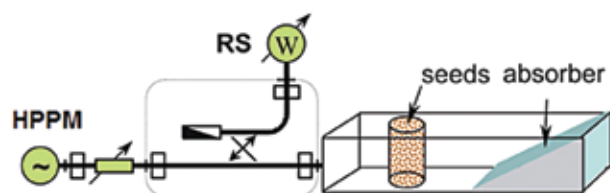
The exact biochemical processes by which microwaves could affect the functioning of living organisms are not very well understood and the mechanism may vary depending upon the amplitude of the field and its frequency and, in the general case, on the duty cycle (Monteiro et al., 2008; Aladjadjiyan, 2010). Yanenko and co-workers (2004) report that among physical methods for the pre-sowing treatment of seeds electromagnetic irradiation deserves special attention. The frequencies of the cell membrane vibrations of biological objects lie in the mm-wave range. Mm-waves are known to be immanent to any living organism. The mm-wave irradiation of biological objects initiates resonant phenomena in living cells and has an overall positive impact on biological organisms (Aladjadjiyan, 2002; Yanenko et al., 2004).

Various experiments showed that HPM can affect plant growth and the prolonged exposure to microwave affects seed germination (Hamada, 2007; Aladjadjiyan, 2010; Salama et al., 2011). Oprica (2008) indicated that microwaves determined variations of catalase and peroxidase activities in *Brassica napus* depending on the microwave exposure time, age of the plants, and condition of seeds (germinated or non-germinated). It was noted that weak intensity of microwaves did not affect plant growth but increased doses slowed seed germination (Oprica, 2008). Ponomarev et al. (1996) studied the direct effect of electromagnetic radiation of the microwave range (wavelength $\lambda = 1$ cm, irradiation time up to 40 min) on the germination of cereals (winter and spring wheat, spring barley, oats). They established increased germination rate in all the tested seeds and reached optimal stimulating effect at 20 min exposure time.

The aim of our study was to evaluate the influence of different frequencies of high power microwave (HPM) pulses on vegetable seed germination and microwave (at 9.3 GHz frequency) effect on seedling growth rate.

Materials and methods

At the Centre of Physical Sciences and Technology, vegetable seeds were irradiated by high power microwaves (HPM) inside the waveguides in 2011. The experimental set-up is shown in Figure 1.



HPPM – a high power pulsed magnetron, RS – a resistive sensor applied as transmitted microwave power meter

Figure 1. Experimental set-up: microwave irradiation on vegetable seeds in waveguide

Special thin wall plastic containers for seed irradiation were used to maintain the uniformity of electric field distribution inside the waveguide. It is supposed that walls of cylindrical container are thin, the humidity of seeds is low, and therefore the electric field

in the container, mounted in the middle of rectangular waveguide, is the same as in the empty waveguide, so can be calculated:

$$E = \sqrt{\frac{2z_B P}{ab}}, \quad z_B = \frac{z_0}{\sqrt{1 - (\lambda/2a)^2}}$$

where Z_B is waveguide impedance, $Z_0 = 377 \Omega$ is free space impedance, a and b are rectangular waveguide dimensions, λ is microwave wavelength.

Table 1 presents the maximum power and electric field level and radiation conditions on seeds in the waveguides.

Table 1. Maximum power and electric field of vegetable seed irradiation in the waveguides

Band	Frequency GHz	Waveguide a × b mm ²	Power kW	E kV m ⁻¹
S	2.6	72 × 34	100	226
C	5.7	40 × 20	100	316
X	9.3	23 × 10	80	606

S, C and X – a strong microwave electric field band

Investigating the effect of microwave exposure, the seeds of vegetables were irradiated by HPM. The main focus was on the thermal heating elimination during seeds irradiation. For that reason short high frequency microwave pulses were used.

The study object was seeds of different harvest years (2000, 2003 and 2008) of radish (*Raphanus sativus* L.) variety 'Babtu žara', tomato (*Lycopersicon esculentum* Mill.) variety 'Viltis', carrot (*Daucus sativus* Rohl.) variety 'Vaiguva' and tomato (*Lycopersicon esculentum* Mill.) variety 'Red Cherry' (seeds harvested in 1980). In the first experiment, all seeds were exposed to microwaves at 9.3 GHz frequency for 10 min, where pulse duration was 4 μ s and pulse repetition – 25 Hz, electric field – 320 kV m⁻¹. In the second experiment, in one treatment the seeds of carrot variety 'Vaiguva' (11 years-old) were exposed to microwaves at 9.3 GHz frequency for 5 and 20 min at 322 kV m⁻¹ electric field. In other treatment, the seeds were exposed to 2.6 GHz microwaves at 241 kV m⁻¹ electric field and to 5.7 GHz microwaves at 329 kV m⁻¹ electric field for 10 min. In both cases pulse duration was 4 μ s and pulse repetition – 25 Hz. Control treatment was non-irradiated seeds. In order to establish HPM effect on seedling growth rate (shoot and root dry weight, plant height, hypocotyl length and height, leaf area, chlorophyll *a*, chlorophyll *b*, chlorophyll *a + b* and carotenoids) tomato (variety 'Viltis'), carrot (variety 'Vaiguva') and radish (variety 'Babtu žara') 3 year-old seeds were irradiated by microwaves at 9.3 GHz frequency for 10 min, where pulse duration was 4 μ s and pulse repetition – 25 Hz, electric field – 320 kV m⁻¹. 14-day old seedlings were used for analysis and measurements.

Determination of seed germination. The seeds were germinated in Petri plates on filtered paper in a thermostat and stored at variable temperature. It means that higher temperature (30°C) was maintained for a 6-hour period and for 18-hour period – temperature was lower (20°C). Samples of 100 seeds each were repeated 4 times. Petri plates were ventilated every day by opening for a few seconds and optimum moisture content was maintained. Radish, carrot, tomato seed germination

energy was observed after 3, 5 and 6 days, respectively. Germination of radish was determined after 7 days and that of carrots and tomatoes after 10 days. Seeds with normally developed rootlet (rootlet diameter was not less than the diameter of the seed) were counted.

Biometric measurements. Plant height, hypocotyl length and height were measured in cm. Fresh mass was measured in g. Dry mass of roots and shoots of vegetable seedlings was evaluated by drying plants in a drying oven ("Venticell MBT", Czech Republic) at 105°C for 24 h. Leaf area was measured by an automatic leaf area meter ("AT Delta-T Devices", England).

Determination of chlorophyll and carotenoids. The amounts of chlorophyll and carotenoids in fresh mass (FM) were determined by spectrophotometry in pure acetone extract (Gavrilenko, Zikalova, 2003), using a "Genesys 6" spectrophotometer ("Thermo Spectronic", USA).

The obtained data were processed using *MS Excel* software (version 7.0) and statistical software *Statistica 7* and *ANOVA*.

Results and analysis

Vegetable yield depends not only on the variety but also on seed quality. The main indicators of good seed quality are purity, germination and germination energy. According to the quality requirements for all seed categories, germination of radish should reach 80%, carrots – 70%, and tomatoes – at least 75% (Bobinas, 1999; Radzevičius et al., 2007). Already in the 1970's Crawford (1977) reported that microwaves can decrease seed germination. He examined the phytotoxic effect of 2450 MHz radiation on the seeds of *Trifolium* and *Medicago* cultivars. All seeds showed a marked decrease in germination after treatment for 15–20 seconds. According to our results (Fig. 2), radish seeds (harvested in 2000) exposed to 9.3 GHz microwaves had higher germination rate (26%), but HPM had no effect on germination energy as compared with non-irradiated seeds (16.7%).

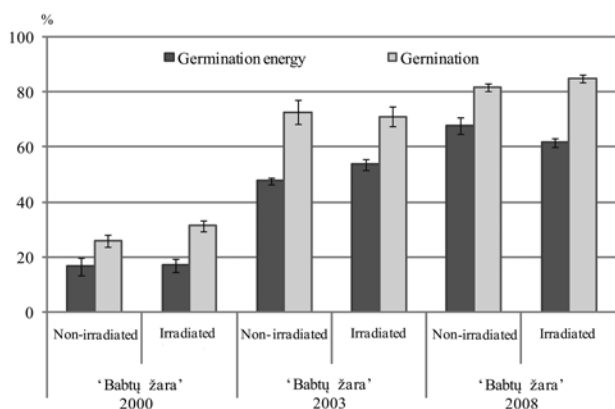


Figure 2. The effect of high power microwave (HPM) irradiation (9.3 GHz) on germination rate and germination energy of radish seeds

Irradiation had no effect on the germination rate of radish seed (harvested in 2003 and 2008) (Fig. 2). HPM increased radish germination energy by 6% in the seeds harvested in 2003, but in the seeds harvested in 2008 there was determined a 6% decrease in germination

energy. Non-thermal effects on seeds and biological objects are due to the direct interaction of the microwaves on molecules or tissue components, since particles seek to orient themselves within the electric field and minimize potential energy (Salles, 1999).

The data (Fig. 3) of this study showed significant increase in germination energy in irradiated tomato seeds harvested in 2003 and 2008 (15.3% and 27.0%, respectively) but irradiation had no effect on tomato germination rate as compared with the control treatments. HPM did not induce germination energy in the tomato (variety 'Viltis') seeds harvested in 2000, but irradiation increased their germination rate by 8.7%. Meanwhile, seeds (harvested in 1980) of the variety 'Red Cherry' had lost their germination and microwave exposure did not induce biological processes. There is opposing information about microwave effect on seed germination. Monteiro and colleagues (2008) have reported about grass seed germination decrease under microwave irradiation. Studies on microwave field effect on the germination rate and the shoot growth rate showed that the microwave fields possibly retard the germination but the effect on the tubule growth is uncertain and the data from the ongoing experiments provided statistically meaningful results (Monteiro et al., 2008). While Raha and co-workers (2011) suggest that high frequency stimulated seed germination for most of the investigated samples as compared to the control.

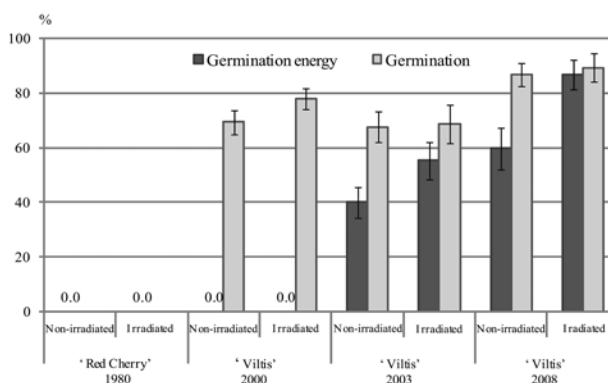


Figure 3. The effect of high power microwave (HPM) irradiation (9.3 GHz) on germination rate and germination energy of tomato seeds

We evaluated the irradiation effect of 9.3 GHz microwave frequency on the seeds of carrot variety 'Vaiguva' (Fig. 4) and found the highest germination rate (19.3%) in the seeds harvested in 2008 and a 4.6% germination rate increase in the seeds harvested in 2003 as compared with non-irradiated.

Microwave exposure significantly increased the germination energy by 3.6% and germination rate by 11.0% in the carrot seeds harvested in 2003. In this case, it was possible that carrot seed molecules might be lifted into the metastable state through the action of electric fields, e.g., when built into a membrane through the high electric field existing there. The high dipole moment of the metastable state implies in general its excitation to be coupled with excitation of homogeneous electric vibrations, i.e. giant dipole oscillations (Frohlich, 1975; Gorny et al., 2007). Evaluation of different HPM frequencies and different effects of irradiation time (Fig. 5) showed that the highest carrot seed germination

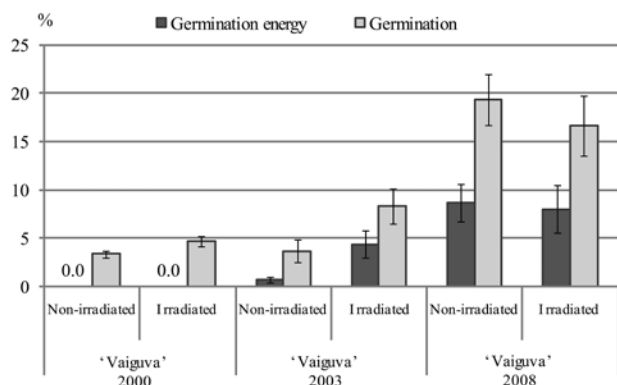


Figure 4. The effect of high power microwave (HPM) irradiation (9.3 GHz) on the germination rate and germination energy of carrot seeds

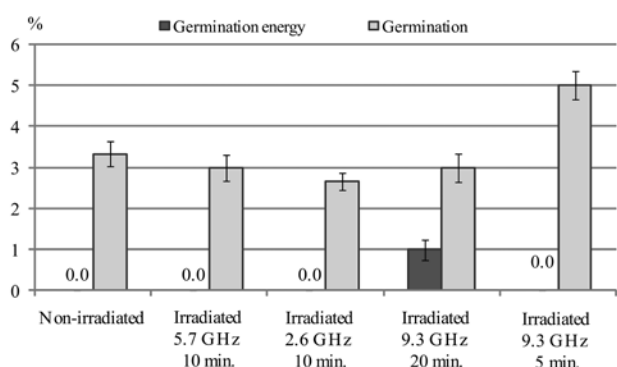


Figure 5. The effect of different high power microwave (HPM) irradiation frequency and time on carrot seeds

rate 5.0% was established at 9.3 GHz microwave frequency under 5 min exposure. A 10-min exposure and 2.6 GHz microwave frequency significantly reduced carrot seed germination by 0.6%. Among other treatment combinations, significant differences were not detected. Various frequencies of HPM had different effect on carrot seed germination. Experiments with different electromagnetic fields' strengths on carrot seed were made in Lithuania in 2007. It was established that different strengths of direct electromagnetic field and exposure time had different effect on carrot yield, quality and seed germination dynamics (Stašelis, 2007).

Seed exposure to HPM (9.3 GHz) had a significant positive effect (1.32 g) on dry weight of tomato (variety 'Viltis') seedling shoots and significant negative effect on root dry mass (0.87 g) of tomato seedlings

(Table 2). Vegetable seedlings growth from irradiated seeds had no significant difference in hypocotyl height. Radish hypocotyl (grown from irradiated seeds) was twice as thick as that of radish grown from non-irradiated seeds. Results indicated that the seed irradiation had significant positive effect on the height of tomato (2.08 cm) and radish (1.76 cm) seedlings, but it had negative influence on the height of carrot seedlings. Irradiated carrot seedlings were shorter (1.0 cm) as compared with non-irradiated. Seeds irradiation determined significantly higher assimilating area (7.3 cm²) in tomato seedlings and significantly lower assimilating area (6.4 cm) in carrot seedlings. Raga and co-workers (2011) observed that irradiation of seeds resulted in a significant increase in plant biomass, but admitted that the effect varied according to the plant species and their ecological adaptation. Other scientists found that direct effect of electromagnetic fields stimulates the development of tomato plants, irradiated tomato sprouts grew up higher, formed more leaves and accumulated more fresh mass (Stašelis et al., 2004). Hamada (2007) investigated different doses of microwaves on wheat and determined that irradiation increased root and shoot lengths also fresh mass in 14 days' old wheat seedlings.

Photosynthetic pigments are very important physiological indexes. In connection with photosynthesis productivity, the amount of photosynthetic pigments can be an evidence of stressors tolerance in plants (Salama et al., 2011; Samuolienė et al., 2012). Our results indicated, that seed exposure to HPM (9.3 GHz) had significant positive effect on the amount of chlorophyll *a* (0.224 mg g⁻¹), chlorophyll *b* (0.048 mg g⁻¹), chlorophylls *a* + *b* (0.272 mg g⁻¹) and carotenoids (0.082 mg g⁻¹) in tomato seedlings fresh mass (Table 3). The opposite effect was determined in carrot seedlings FM. Seed irradiation did not influence the amount of photosynthetic pigments in radish seedlings FM. Positive effect of HPM on photosynthetic pigment amount in wheat seedlings was reported by Hamada (2007). He admitted that chlorophyll *a/b* and carotenoids ratios increased in 7 days' old seedlings, but they tended to diminish in 14 days' old seedlings as compared with non-irradiated seedlings. The possible explanation 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 (Aladjadjiyan, 2002).

Table 2. The effect of seed irradiation (9.3 GHz) on the growth parameters of tomato, carrot and radish seedlings

Treatment	Dry weight g				Hypocotyl height cm		Hypocotyl thickness cm		Plant height cm		Assimilating area cm ²	
	Roots	STDEV	Shoots	STDEV	height	STDEV	thickness	STDEV	height	STDEV	area	STDEV
Tomato C.	3.50	0.32	1.66	0.32	5.86	0.53	0.10	0.00	11.42	1.07	15.5	2.51
Tomato Ir.	2.63	0.10	2.98	0.42	5.92	0.48	0.10	0.00	13.50	0.99	22.8	2.63
Carrots C.	12.72	0.85	7.91	1.07	4.40	1.67	0.10	0.01	14.90	0.22	22.1	2.66
Carrots Ir.	12.70	0.92	7.02	1.49	3.20	1.59	0.30	0.09	13.90	0.39	15.7	2.20
Radish C.	5.47	0.68	5.20	0.49	2.16	0.19	0.74	0.11	12.70	0.66	101.5	5.68
Radish Ir.	5.07	0.53	5.26	0.55	2.38	0.18	1.34	0.14	14.46	0.55	108.1	7.13

STDEV – standard deviation, C. – control, non-irradiated, Ir. – irradiated seeds

Table 3. The effect of seed irradiation (9.3 GHz) on photosynthetic pigments in tomato, carrot and radish seedlings

Treatment	Chlorophyll <i>a</i> mg g ⁻¹ FM	STDEV	Chlorophyll <i>b</i> mg g ⁻¹ FM	STDEV	Chlorophylls <i>a</i> + <i>b</i> mg g ⁻¹ FM	STDEV	Carotenoids mg g ⁻¹ FM	STDEV
Tomato C.	0.557	0.009	0.291	0.023	0.848	0.049	0.146	0.014
Tomato Ir.	0.781	0.085	0.339	0.021	1.120	0.114	0.228	0.027
Carrots C.	1.224	0.040	0.456	0.013	1.681	0.051	0.349	0.018
Carrots Ir.	1.090	0.043	0.412	0.014	1.502	0.033	0.324	0.021
Radish C.	0.616	0.067	0.290	0.051	0.906	0.108	0.140	0.021
Radish Ir.	0.618	0.060	0.286	0.034	0.904	0.067	0.152	0.027

Explanations under Table 2

Studies related to the HPM on the biological objects are a new and dynamic field for scientific research, involving plant biotechnology and microwave engineering. More studies in this area need to be done. However, our study showed that we can expect new promising results in the experiments with HPM and biological objects.

Conclusions

1. Radish seeds (variety 'Babtų žara') harvested in 2000 and exposed to 9.3 GHz microwaves had higher germination as compared with non-irradiated seeds. High power microwaves (HPM) increased radish germination energy by 6% in seeds harvested in 2003.

2. HPM exposure significantly increased the germination energy in tomato seeds (variety 'Viltis') harvested in 2003 and 2008.

3. Microwave exposure significantly increased the germination energy and germination in 8 year-old carrot seeds. The highest carrot seed germination was established at 9.3 GHz microwave frequency under 5 min exposure.

4. Seed exposure to HPM (9.3 GHz) had a significant positive effect on dry weight of tomato (variety 'Viltis') seedling shoots and on tomato and radish (variety 'Babtų žara') seedling height, but it had a negative influence on carrot (variety 'Vaiguva') seedling height.

5. Seed irradiation with HPM (9.3 GHz) had a significant positive effect on the amount of chlorophyll *a*, chlorophyll *b*, chlorophylls *a* + *b* and carotenoids in tomato seedlings (variety 'Viltis') fresh mass, but the opposite effect was determined in carrot (variety 'Vaiguva') seedlings fresh mass.

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Didelės galios mikrobangų impulsų poveikis: (1) daržo augalų sėklų daigumui ir daigų augimo rodikliams

A. Radzevičius¹, S. Sakalauskienė¹, M. Dagys², R. Simniškis², R. Karklelienė¹, Č. Bobinas¹, P. Duchovskis¹

¹Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės institutas

²Fizinių ir technologijos mokslų centras

Santrauka

Tyrimo metu vertintas didelės galios mikrobangų poveikis augalų sėklų daigumui ir daigams. Daržovių sėklos buvo veikiamos aukšto dažnio mikrobangomis (ADM). Tyrimo metu daugiausia dėmesio skirta mikrobangų šiluminio poveikio eliminavimui švitinant augalų sėklas, todėl naudoti trumpi, galingi, retai pasikartojantys mikrobangų impulsai. Tyrimo objektas – valgomojo ridikėlio (*Raphanus sativus* L.) veislės ‘Babtų žara’, valgomosios morkos (*Daucus sativus* Rohl.) veislės ‘Vaiguva’ bei valgomojo pomidoro (*Lycopersicon esculentum* Mill.) veislės ‘Viltis’ 2000, 2003 bei 2008 m. derliaus sėklos ir valgomojo pomidoro (*Lycopersicon esculentum* Mill.) veislės ‘Red Cherry’ 1980 m. derliaus sėklos. Pirmojo bandymo metu visos sėklos 10 min paveiktos 9,3 GHz dažnio mikrobangomis. Antrojo bandymo metu veislės ‘Vaiguva’ 2000 m. derliaus morkų sėklos vienu atveju spinduliuotos 9,3 GHz mikrobangomis 5 ir 10 min, kitu atveju mikrobangų dažniai buvo pakeisti į 2,6 ir 5,7 GHz, o švitinimo trukmė – 10 min. Siekiant nustatyti mikrobangų poveikį daigų augimo rodikliams, 2008 m. derliaus pomidorų, morkų ir ridikėlių sėklos 10 min paveiktos 9,3 GHz dažnio mikrobangomis. Tyrimų metu nustatyta, kad 2000 m. derliaus ridikėlių sėklos, pašvitintos 9,3 GHz mikrobangomis, pasižymėjo geresniu daigumu, lyginant su nešvitintomis. ADM 2003 m. derliaus ridikėlių sėklų dygimo energiją padidino 6 %. Esminis teigiamas mikrobangų poveikis užfiksuotas veislės ‘Viltis’ pomidorų 2003 ir 2008 m. derliaus sėklų dygimo energijai. ADM iš esmės padidino 2003 m. derliaus morkų sėklų daigumą ir dygimo energiją. Didžiausias morkų sėklų daigumas nustatytas jas 5 min paveikus 9,3 GHz mikrobangomis. Sėklų apšvitinimas (9,3 GHz) turėjo esminį teigiamą poveikį pomidorų daigų antžeminės dalies sausosios masės kiekiui ir pomidorų bei ridikėlių daigų aukščiui, tačiau neigiamai paveikė morkų daigų aukštį. Sėklų švitinimas ADM (9,3 GHz) turėjo esminę teigiamą įtaką chlorofilo *a*, chlorofilo *b*, chlorofilų *a + b* ir karotenoidų kiekiui pomidorų daigų lapuose, tačiau morkų daigų lapuose nustatytas priešingas poveikis – fotosintezės pigmentų kiekio sumažėjimas.

Reikšminiai žodžiai: *Daucus sativus*, fotosintezės pigmentai, *Lycopersicon esculentum*, *Raphanus sativus*, švitinimas.