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Enhancing Zinnia (*Zinnia elegans* Jacq.) Seed Quality through Microwaves Application

Dorota Szopińska and Hanna Dorna *

Department of Phytopathology, Seed Science and Technology, Poznań University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland; dorota.szopinska@up.poznan.pl

* Correspondence: hanna.dorna@up.poznan.pl

Abstract: In organic farming, microwave irradiation can be used as an alternative to standard chemical seed treatment. The increase in temperature during this treatment may negatively affect the germination of low-quality seeds. The aim of the study was to evaluate the effect of microwaves on germination and the health of zinnia seeds using four seed samples varying in initial seed quality. Seeds were placed in a beaker with distilled water and irradiated at power output levels of 500, 650 and 750 W for 30, 35, 40, 45 and 50 s. Controls were untreated seeds, seeds treated with fungicide and seeds soaked in water for 30, 35, 40, 45 and 50 s. Germination and health tests were performed for controls and microwave-treated seeds. Analyzed samples differed in seed quality and response to microwave irradiation. Improvement in seed germination after microwave treatment was observed in three of the tested samples. The fungi *Alternaria alternata*, *A. zinniae* and *Fusarium* spp. prevailed on the seeds. Microwave treatment, especially at power outputs of 650 and 750 W, usually positively affected seed health, significantly increasing the number of seeds free of fungi, but when water temperature during treatment exceeded 60 °C significant deterioration in germination parameters was observed in all tested samples.

Keywords: *Alternaria alternata*; *Alternaria zinniae*; *Fusarium* spp.; zinnia seeds; germination; microwave treatment

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1. Introduction

Common zinnia (*Zinnia elegans* Jacq.), an annual ornamental plant from the Asteraceae family, is appreciated and cultivated worldwide for its long-term flowering and wide color range. The ornamental value and growth of zinnia are frequently affected by seed-borne pathogens. The fungi *Alternaria alternata* (Fr.) Keissler, *A. zinniae* M.B. Ellis, *Botrytis cinerea* Pers., *Cladosporium* spp. and *Fusarium* spp. have often been detected on zinnia seeds [1,2]. Among them, *A. zinniae* is considered the most important pathogen of this plant, responsible for spotting of petals, foliage and stems, as well as rotting of roots [3,4]. All lots of zinnia seeds may be contaminated with this pathogen in some years [personal observation]. High seed infestation with fungi, especially from genera *Alternaria* and *Fusarium*, can also negatively affect zinnia seed germination [4–6]. Synthetic chemicals are usually used against seed-borne fungi; however, most of them are not an option for organic growers. Therefore, numerous alternative physical methods have been proposed, including the use of thermal energy (e.g., hot water, dry heat, aerated steam) and various types of radiation (e.g., gamma rays, microwaves, ultrasonic, UV) to control these pathogens in organic farming [7,8].

Microwaves are electromagnetic waves with frequencies (wavelengths) ranging from 300 MHz (λ = 1 m) up to 300 GHz (λ = 1 mm) [9]. They are widely used in the food industry, communication, medicine and metallurgy. Microwave radiation has also been successfully applied in agriculture, to reduce seed infestation by pathogenic microorganisms. Microwave processing was proposed, for example, as a potential non-chemical method to control seed-borne *Ascochyta lentis* Vassilievsky and *Botrytis cinerea* in lentils (*Lens culinaris* Medik.) [10,11], *Fusarium graminearum* Schwabe in wheat (*Triticum aestivum* L.) [12] and *Ustilago nuda* (C.N. Jensen) Kellerm. & Swingle in barley (*Hordeum vulgare* L.) [13]. During microwave radiation, the death of fungal cells occurs mainly as a result of temperature increase. The interaction between polar molecules of water and the microwaves causes the rotation of water molecules at a speed that generates heat [14]. This leads to the denaturation of proteins, enzymes and nucleic acids. Bonds holding proteins are destroyed, and enzymatic activities necessary for carrying out metabolic processes may be at risk [14–16]. Moreover, some non-thermal effects of microwaves, such as the formation of pores in a cellular membrane due to their interaction with polar molecules (electroporation), may cause further deterioration in seed-associated microorganisms [15,17]. The formation of these pores leads to the leakage of cellular content outside, including DNA [14].

The thermal effect is a function of microwave power and energy outputs, wave forms, modulation and time of exposure [17,18]. An increase in temperature, as the consequence of treatment prolongation and/or an increase in power output, also leads finally to the deterioration of seed viability. The loss of seed germination is considered the major limitation of microwave treatment application [19,20]. However, there are also numerous reports that properly applied microwave irradiation can improve seed germination and plant emergence [21–27]. Knox et al. [28] linked the deterioration of wheat seeds' viability, as a result of microwave treatment, with a decrease in seed moisture content. To avoid this problem, an increase in water content in seeds before treatment is recommended—as, at a higher moisture content, most of the power is absorbed by the free water in the seeds, thus resulting in a reduction in cell damages [12,29].

Our preliminary experiments showed that the exposition of dry zinnia seeds to microwave treatment as well as increasing water content in the seeds before treatment had equally detrimental effects on zinnia seed germination (data not published). The other promising option seems to be immersing seeds in water during microwave treatment. Our previous experiment showed that this procedure to some extent protected carrot seeds from injuries caused by overheating [27]. Moreover, thermotherapy, based on hot water treatment, has already been proven to be efficient for the control of *A. zinniae* on zinnia seeds [30]. Microwave treatment compared with standard hot water treatment is fast, simple and does not require constant temperature control; however, like any thermotherapy, it may negatively influence the germination of low-quality seeds [8]. Therefore, in the present experiment four zinnia seed samples, varying in initial seed quality, were tested. The effect of microwave irradiation, combined with hot water treatment, on seed germination and infestation with fungi was evaluated to establish the optimal conditions for zinnia seed treatment.

2. Materials and Methods

The experiment was performed at the Department of Phytopathology, Seed Science and Technology of the Poznań University of Life Sciences in Poland.

Four commercially available zinnia seed samples varying in seed germination and infestation with fungi were used in the experiment. All samples (sample I—cv. Jowita, lot No 304/63/51/151A; sample II—cv. Jowita, lot No 804/63/51/277A; sample III—cv. Illumination, lot No 630/64/13/135/520; sample IV—cv. Orange King, lot No 704/63/51/835/A) were obtained from Torseed Seed Company in Toruń (Poland). The seeds were treated in Microwave oven type M593 (microwave frequency 2.45 GHz, wavelength 12.24 cm).

For treatment, 2 g of seeds were placed in a glass beaker with a capacity of 250 mL filled with distilled water to a volume of 100 mL. Then, the beaker was located centrally in the microwave oven and exposed to microwave radiation at three different output levels, 500, 650 and 750 W, for 30, 45, 40, 45 and 50 s. Directly after treatment, the seeds were transferred into a sieve and cooled immediately in distilled water and then under running tap water for 2 min and dried for 48 h at 20 °C and 45% RH. Additionally, directly after each treatment, the water temperature was measured (three times for each sample; 12

measurements in total for each treatment). The same procedure of washing and drying was performed for the seeds soaked in distilled water for 30, 35, 40, 45 and 50 s but not exposed to microwave radiation (water control). To compare the effectiveness of microwave radiation with standard chemical treatment, seeds were treated with fungicide Zaprawa Nasienna T 75 DS/WS (a.i. thiram—75%) at a dose of 5 g kg^{-1} of seeds (chemical control).

For the untreated seeds (control), seeds soaked in water, seeds treated with fungicide and microwave-treated seeds, germination and health tests were performed. Seed germination was evaluated on 300 seeds from each treatment (six replicates of 50 seeds). Seeds were placed in 9 cm diameter Petri dishes (25 seeds per dish) on six layers of blotter paper moistened with distilled water and then incubated for 10 days at 20 °C in darkness. After 5 and 10 days of incubation, germination at the first and final counts (the percentage of normal seedlings) was evaluated, respectively. Moreover, after 10 days, the percentages of abnormal diseased seedlings, deformed seedlings and ungerminated fresh and dead seeds were determined according to the International Seed Testing Association (ISTA) Rules [31]. The deep-freeze-blotter test was applied for seed health analysis [1]. For each treatment, 200 seeds, i.e., 4 replicates of 50 seeds, were tested. The seeds were placed in 9 cm diameter Petri dishes (10 seeds per dish) on six layers of blotter paper moistened with distilled water. The seeds were incubated in darkness at 20 °C for 24 h and frozen at −20 °C for 24 h and then for 8 days at 20 °C under 12 h alternating cycles of NUV light and darkness. After incubation, seeds were examined under a stereomicroscope (magnification ×80) for fungal growth and sporulation [32,33]. The percentages of seeds infested with individual fungi and seeds free of fungi were determined.

All parameters describing seed germination and infestation with fungi were evaluated by one-way analysis of variance followed by Duncan's multiple range test, at a level $\alpha = 0.05$.

3. Results

3.1. Water Temperature

Fluctuations in water temperature between measurements, for each treatment variant, ranged from 1.0 to 4.5 °C (Table 1). With the increase in irradiation time, regardless of power output, the average water temperature successively increased. In the case of the lowest power output (500 W), the temperature rose up from 44.0 to 53.2 °C. If microwaves at 650 W power output were applied, the temperature increased from 48.7 to 62.4 °C, whereas, when the highest (750 W) output power was used, the temperature ranged from 52.3 to 64.2 °C.

Table 1. Water temperature measured after microwave treatment.

Input Power (W)	Treatment Time (s)	Temperature (°C)		
		Min.	Max.	Average
500	30	43.0	45.0	44.0
	35	44.0	48.0	46.3
	40	45.0	48.0	46.7
	45	48.0	49.0	48.4
	50	50.0	54.0	53.2
650	30	47.0	51.0	48.7
	35	49.0	52.5	51.6
	40	51.5	55.0	52.9
	45	54.0	57.0	54.8
	50	61.0	64.0	62.4
750	30	51.0	54.0	52.3
	35	54.0	57.0	55.9
	40	57.0	58.0	57.6
	45	58.0	60.0	59.8
	50	63.0	65.0	64.2

3.2. Seed Germination

All analyzed untreated seed samples were characterized by relatively low seed germination, both at the first and at the final count, and a very high percentage of abnormal diseased seedlings and dead seeds (Tables 2–5). The highest percentage of germinating seeds at the final count showed seeds of sample I (54.7%) followed by seeds of sample II, III and IV (44.7, 32.3 and 31.3%, respectively). The effects of microwave irradiation on seed germination varied significantly between samples, but each time that water temperature during treatment exceeded 60 °C (i.e., when seeds were exposed to microwave radiation at output powers of 650 and 750 W for 50 s), significant deterioration in the germination parameters was observed, regardless of initial sample quality. Soaking in water for 45 s also negatively affected seed germination at the first and at the final counts in sample I. Nevertheless, improvement in these parameters, associated with the decrease in the number of abnormal seedlings, was observed when irradiation at 650 W power output was applied for 30, 35 and 40 s, and when seeds of this sample were irradiated at the power output of 750 W for 30 s (Table 2).

Table 2. The effect of microwave treatment on zinnia seed germination—sample I.

Treatment	Treatment Time (s)	Germination at the First Count (%)		Germination at the Final Count (%)		Abnormal Diseased Seedlings (%)		Dead Seeds (%)	
Control [1]	-	52.7	b–e [2]	54.7	c–g	40.0	a–e	5.3	c–e
Fungicide	-	51.0	c–f	59.3	a–e	29.7	e–h	9.7	b–d
Distilled water	30	51.0	c–f	51.7	d–h	38.7	a–f	9.7	bc
	35	61.3	bc	62.7	a–c	27.7	gh	5.3	de
	40	41.3	fg	49.0	e–h	42.7	a–c	7.0	c–e
	45	37.7	g	42.3	hi	49.3	a	7.3	c–e
	50	42.3	e–g	45.7	gh	47.0	a	7.3	c–e
MW 500 W	30	46.7	e–g	49.0	e–h	45.7	ab	6.3	c–e
	35	46.0	e–g	48.0	f–h	45.0	ab	6.7	c–e
	40	47.7	e–g	49.7	e–h	46.0	ab	4.0	e
	45	49.7	d–f	53.3	c–g	41.7	a–d	5.0	de
	50	58.0	a-d	62.0	a–d	31.0	d–h	7.0	c–e
MW 650 W	30	64.7	a	67.0	ab	26.7	gh	5.7	c–e
	35	68.3	a	69.0	a	24.7	h	4.7	de
	40	63.7	a	64.0	a–c	31.3	d–h	3.3	e
	45	60.3	a–c	60.7	a–d	32.3	c–h	7.0	c–e
	50	25.7	h	30.0	j	41.3	a–d	16.7	b
MW 750 W	30	66.3	a	66.0	ab	28.0	f–h	6.0	c–e
	35	62.7	ab	63.3	a–c	25.7	gh	10.0	b–d
	40	59.7	a–d	62.7	a–c	26.3	gh	10.0	b–d
	45	44.7	e–g	48.0	f–h	35.3	b–g	15.3	b
	50	25.0	h	35.7	ij	27.0	gh	30.7	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500 W, MW 650 W, MW 750 W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Generally, applied microwave treatments, if water temperature ranged from 44 to 60 °C, did not negatively affect seed germination in sample II. However, soaking seeds from this sample in distilled water for 35 and 40 s resulted in a significant increase in the percentage of abnormal diseased seedlings and a decrease in seed germination, especially at the first count (Table 3).

Table 3. The effect of microwave treatment on zinnia seed germination—sample II.

Treatment	Treatment Time (s)	Germination at the First Count (%)		Germination at the Final Count (%)		Abnormal Diseased Seedlings (%)		Dead Seeds (%)	
Control [1]	-	44.3	a–c [2]	44.7	a–e	40.0	c–g	15.3	c–f
Fungicide	-	51.3	a	52.0	a	34.3	g–i	9.7	ef
Distilled water	30	37.3	c–f	40.0	c–f	44.7	a–e	14.3	d–f
	35	32.7	ef	37.7	d–f	47.3	ab	15.3	c–e
	40	31.3	f	32.7	fg	50.3	a	17.0	b–d
	45	42.7	a–d	43.0	a–e	40.7	b–g	16.0	b–d
	50	40.7	b–e	40.0	b–f	47.7	a–c	12.3	d–f
MW 500 W	30	38.3	b–f	38.7	d–f	41.7	a–g	19.0	b–d
	35	38.7	b–f	38.7	d–f	44.3	a–f	16.7	b–d
	40	33.7	d–f	36.0	e–g	48.3	a–d	14.3	d–f
	45	50.3	a	50.7	ab	39.7	c–g	9.0	f
	50	44.3	a–c	44.7	a–e	35.7	e–h	16.3	b–d
MW 650 W	30	39.0	b–f	40.7	b–f	38.3	e–h	21.0	b–d
	35	39.7	b–f	40.7	b–f	39.3	d–g	20.0	b–d
	40	48.7	ab	49.0	a–c	37.0	e–h	14.0	d–f
	45	42.7	a–d	45.0	a–e	33.3	g–i	21.3	b–d
	50	24.0	g	29.0	g	25.3	i	45.7	a
MW 750 W	30	47.7	ab	48.3	a–d	35.0	f–h	15.3	c–e
	35	47.7	ab	48.7	a–d	29.0	hi	19.0	b–d
	40	40.7	b–e	41.3	b–f	32.3	g–i	24.0	bc
	45	37.7	c–f	38.3	d–f	33.0	g–i	24.0	bc
	50	7.7	h	8.7	h	32.0	g–i	50.3	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500W, MW 650 W, MW 750 W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Applied microwave treatments largely improved the germination of sample III seeds (Table 4). An increase in seed germination at the first and the final counts was recorded for seeds irradiated at 500 W for 30, 40, 45 and 50 s, when a radiation of 650 W was applied for 30–45 s, and if seeds were irradiated at 750 W for 30–40 s. Moreover, improvement in germination at the final count was observed for seeds irradiated at 500 W power output for 35 s. The enhancement of seed germination at the first and the final counts was frequently associated with a significant decrease in the percentage of abnormal diseased seedlings. Fungicide treatment also improved seed germination; however, microwave-treated seeds were characterized by frequently higher values of germination at the first and final counts. Soaking in water generally did not affect the germination parameters of sample III seeds.

Treating seeds of sample IV with fungicide as well as soaking them in distilled water for 30–50 s positively affected seed germination at the first and the final counts. A beneficial effect of microwave treatment on seed germination at the first and the final counts, associated with a significant decrease in the percentage of abnormal diseased seedlings, was observed for seeds irradiated at 500 W for 35–50 s, at 650 W for 30–45 s and at 750 W for 30–40 s. Moreover, a significant increase in the percentage of normal seedlings at the final count was observed for seeds treated with microwaves at a power output of 500 W for 30 s and of 750 W for 45 s.

Table 4. The effect of microwave treatment on zinnia seed germination—sample III.

Treatment	Treatment Time (s)	Germination at the First Count (%)		Germination at the Final Count (%)		Abnormal Diseased Seedlings (%)		Dead Seeds (%)	
Control [1]	-	29.7	f–h [2]	32.3	g–i	49.7	a	18.0	d
Fungicide	-	37.7	de	41.7	b–f	39.3	a–g	14.7	d
Distilled water	30	34.3	e–g	36.0	e–h	46.0	a–d	17.3	d
	35	37.3	d–f	40.0	c–g	45.7	a–d	14.0	d
	40	34.3	e–g	38.3	d–h	46.3	a–c	15.3	d
	45	34.3	e–g	36.0	e–h	48.7	ab	15.0	d
	50	31.7	e–g	35.3	f–h	47.0	a–c	17.3	d
MW 500 W	30	45.7	b–d	47.3	b–d	39.0	a–g	13.3	d
	35	37.0	d–f	42.3	b–f	37.3	c–g	19.0	d
	40	47.7	a–c	50.7	ab	30.7	gh	18.7	d
	45	45.7	b–d	49.3	a–c	38.0	b–g	12.3	d
	50	39.0	c–e	45.0	b–e	40.0	a–g	15.0	d
MW 650 W	30	45.7	a	58.7	a	24.0	h	14.0	d
	35	43.7	b–d	45.7	b–e	42.0	a–f	11.7	d
	40	49.3	ab	51.0	ab	33.7	e–h	14.7	d
	45	48.0	a–c	51.0	ab	34.0	d–g	15.0	d
	50	24.3	h	27.0	i	29.7	gh	38.7	b
MW 750 W	30	44.7	b–d	47.0	b–d	39.0	a–g	13.7	d
	35	44.7	b–d	45.3	b–e	37.3	c–g	17.3	d
	40	42.7	b–d	45.0	b–e	37.3	c–g	16.7	d
	45	27.3	gh	30.0	hi	43.0	a–e	27.0	c
	50	4.7	i	5.3	j	32.0	f–h	61.7	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500 W, MW 650 W, MW 750 W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Table 5. The effect of microwave treatment on zinnia seed germination—sample IV.

Treatment	Treatment Time (s)	Germination at the First Count (%)		Germination at the Final Count (%)		Abnormal Diseased Seedlings (%)		Dead Seeds (%)	
Control [1]	-	30.0	fg [2]	31.3	f	45.0	a	23.7	b–f
Fungicide	-	48.7	a–d	51.0	a–d	23.7	d	20.3	d–f
Distilled water	30	43.3	b–e	44.7	b–e	31.7	b–d	23.0	b–f
	35	39.7	de	43.3	c–e	38.7	ab	18.0	f
	40	43.7	b–e	47.7	a–e	30.7	b–d	21.7	b–f
	45	53.7	a–c	54.0	a–c	25.7	d	19.3	ef
	50	50.0	a–d	51.7	a–d	27.3	cd	21.0	c–f
MW 500 W	30	37.3	ef	41.0	de	36.3	a–c	22.7	b–f
	35	45.7	a–e	49.0	a–d	24.3	d	26.7	b–e
	40	45.7	a–e	46.7	a–e	27.0	cd	26.3	b–e
	45	44.3	a–e	45.0	b–e	29.0	b–d	25.0	b–f
	50	43.3	b–e	46.3	a–e	33.3	b–d	20.3	d–f
MW 650 W	30	47.0	a–e	49.0	a–d	30.7	b–d	20.0	d–f
	35	44.0	a–e	46.0	a–e	29.7	b–d	24.3	b–f
	40	42.7	c–e	44.0	c–e	28.3	b–d	27.7	b–d
	45	40.3	de	44.0	c–e	30.7	b–d	25.3	b–f
	50	27.7	g	37.0	ef	33.3	b–d	29.3	b

Table 5. *Cont.*

Treatment	Treatment Time (s)	Germination at the First Count (%)		Germination at the Final Count (%)		Abnormal Diseased Seedlings (%)		Dead Seeds (%)	
MW 750 W	30	54.3	ab	55.3	ab	24.3	d	19.7	ef
	35	54.7	a	56.0	a	22.7	d	21.3	b–f
	40	42.7	c–e	44.0	c–e	25.7	d	28.7	bc
	45	36.3	fe	41.7	de	27.7	cd	27.3	b–e
	50	5.3	h	12.0	g	26.3	cd	52.7	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500 W, MW 650 W, MW 750W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level α = 0.05, according to Duncan's multiple range test.

3.3. Seed Health

On the seeds of the tested samples, fungi from genera *Alternaria* and *Fusarium* prevailed (Tables 6–9). In sample I, *A. alternata* and *Fusarium* spp. infested 92.0 and 91.0% of untreated seeds, respectively, while *A. zinniae* was detected on 26.5% of seeds (Table 6). Fungicide treatment controlled *A. alternata* and *Fusarium* spp. to some extent but was not effective against *A. zinniae*. Soaking seeds in distilled water for 30–45 s did not affect seed contamination with fungi; however, when seeds were soaked for 50 s, some increase in their infestation with *A. alternata* and *Fusarium* spp. was observed. Treatment with microwave radiation at power output levels of 650 and 750 W, regardless of treatment time, resulted in a significant decrease in the percentage of seeds infested with *A. alternata* and *Fusarium* spp. and a significant increase in the percentage of seeds free of fungi. Microwave radiation at a power output of 500 W was also effective against these fungi, but only if applied for time longer than 35 s. Reduction in seed infestation with *A. zinniae* was observed only in seeds treated with microwave radiation at 650 W power output for 35 s and in seeds exposed to microwave radiation at 750 W for 35–50 s.

Untreated seeds in sample II were characterized by high seed infestation with both *A. alternata* and *A. zinniae* —72.0 and 77.5%, respectively—and 64.0% infestation with *Fusarium* spp. (Table 7). Soaking in water mostly did not affect the presence of *Alternaria* spp. on zinnia seeds, but in few cases it increased seed infestation with *Fusarium* spp. Treating seeds with fungicide as well as exposing them to microwave radiation, regardless of power output and treatment time, significantly increased the number of seeds free of fungi and decreased the percentage of seeds infested with *Alternaria* spp. and *Fusarium* spp. The lowest numbers of seeds infested with these fungi were observed after treatment with microwave radiation at power output levels of 650 and 750 W for 40–50 s.

Alternaria alternata, *A. zinniae* and *Fusarium* spp. infested 76.5, 71.5 and 66.5% of untreated seeds in sample III, respectively (Table 8). Fungicide significantly reduced seed contamination with these fungi; however, microwave treatment was frequently most effective, especially in *A. zinniae* control. Soaking in water for 40 s also decreased seed infestation with *A. alternata*, and soaking for 30, 40, 45 and 50 s reduced the percentage of seeds infested with *A. zinniae*, but not as efficiently as microwave treatment. Seeds soaked in water for 30, 40 and 54 s, on the other hand, were characterized by higher seed infestation with *Fusarium* spp. Nevertheless, exposure of seeds to microwave radiation at 500 W for 50 s, at 650 W for 35, 45 and 50 s, and at 750 W for 35–50 s, significantly decreased seed infestation with these fungi. All microwave treatments, except 30 s exposition to microwave radiation at a power output of 500 W, significantly increased the number of seeds free of fungi in relation to untreated seeds and water control.

Table 6. The effect of microwave treatment on the health of zinnia seeds—sample I.

Treatment	Treatment Time (s)	Percent of Seeds Infested with						Seeds Free of Fungi (%)	
		Alternaria alternata		*Alternaria zinniae*		*Fusarium* spp.			
Control [1]	-	92.0	b [2]	26.5	a–d	91.0	a–c	0	i
Fungicide	-	65.5	fg	20.0	b–g	60.5	g–i	1.5	gh
Distilled water	30	94.5	ab	27.5	a–d	95.0	ab	0	i
	35	87.0	b–d	20.5	b–g	85.5	cd	0	i
	40	90.0	bc	27.0	a–d	90.0	bc	0	i
	45	89.5	bc	30.5	ab	87.5	cd	0	i
	50	98.0	a	26.0	a–e	96.0	a	0	i
MW 500 W	30	92.5	bc	24.5	a–f	81.0	de	0.5	hi
	35	87.0	bc	30.5	ab	83.5	cd	0	i
	40	84.5	cd	29.0	a–c	74.0	ef	1.0	hi
	45	72.5	ef	27.0	a–d	64.5	f–h	4.0	g
	50	56.5	g	25.0	a–e	42.5	jk	17.0	de
MW 650 W	30	78.0	de	34.5	a	72.0	e–g	0.5	hi
	35	62.0	fg	14.0	g	53.0	h–j	10.0	f
	40	62.5	fg	19.5	c–g	61.0	g–i	13.0	ef
	45	60.0	g	18.0	d–g	51.0	i–k	16.0	de
	50	15.0	i	5.5	h	7.5	lm	66.5	b
MW 750 W	30	60.0	g	18.0	d–g	49.5	i–k	8.0	f
	35	54.5	g	16.5	e–g	40.5	k	22.0	d
	40	27.0	h	15.0	fg	15.0	l	53.0	c
	45	13.0	i	6.0	h	6.5	mn	72.0	b
	50	2.0	j	4.0	h	3.0	n	84.5	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500 W, MW 650 W, MW 750 W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Table 7. The effect of microwave treatment on the health of zinnia seeds—sample II.

Treatment	Treatment Time (s)	Percent of Seeds Infested with						Seeds Free of Fungi (%)	
		Alternaria alternata		*Alternaria Zinniae*		*Fusarium* spp.			
Control [1]	-	72.0	a [2]	77.5	Ab	64.0	c	0	i
Fungicide	-	24.5	d–f	39.5	Gh	23.0	fg	20.5	ef
Distilled water	30	70.0	a	68.0	Bc	71.5	bc	0	i
	35	74.0	a	66.5	Cd	81.5	a	0	i
	40	69.0	a	72.0	a–c	78.0	ab	0	i
	45	72.0	a	69.5	a–c	64.5	c	0	i
	50	77.0	a	78.0	A	75.0	ab	0	i
MW 500 W	30	52.0	b	56.5	De	43.0	d	7.0	h
	35	39.5	c	46.5	e–g	40.0	de	6.5	h
	40	38.5	c	51.5	Fg	38.5	de	10.0	gh
	45	36.0	cd	49.0	e–g	37.0	de	16.5	fg
	50	34.5	cd	44.5	Fg	33.0	d-f	19.0	f
MW 650 W	30	33.5	c–e	48.5	e–g	34.5	de	11.0	gh
	35	31.0	c–f	44.0	Fg	34.5	de	21.0	ef
	40	22.0	ef	39.0	Gh	21.5	g	29.5	de
	45	21.0	f	31.0	Hi	30.0	e–g	36.5	cd
	50	4.5	h	23.5	Ij	6.0	h	65.0	a

Table 7. *Cont.*

Treatment	Treatment Time (s)	Percent of Seeds Infested with						Seeds Free of Fungi (%)	
		Alternaria alternata		*Alternaria Zinniae*		*Fusarium* spp.			
MW 750 W	30	32.5	c–f	40.5	f–h	20.0	g	24.5	ef
	35	28.5	c–f	38.5	Gh	19.5	g	21.5	ef
	40	12.5	g	22.0	Ij	7.5	h	54.0	b
	45	8.0	g	27.5	I	7.5	h	45.5	bc
	50	2.0	h	17.0	J	5.5	h	64.5	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500 W, MW 650 W, MW 750 W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level α = 0.05, according to Duncan's multiple range test.

Table 8. The effect of microwave treatment on the health of zinnia seeds—sample III.

Treatment	Treatment Time (s)	Percent of Seeds Infested with						Seeds Free of Fungi (%)	
		Alternaria alternata		*Alternaria Zinniae*		*Fusarium* spp.			
Control [1]	-	76.5	a [2]	71.5	A	66.5	cd	0	g
Fungicide	-	20.5	fg	32.0	c	26.5	fg	26.0	d
Distilled water	30	72.0	a	28.5	c	84.5	a	0	g
	35	68.5	ab	63.5	ab	73.5	bc	0	g
	40	59.5	bc	38.0	c	84.5	a	0	g
	45	67.5	ab	57.0	b	85.0	a	0	g
	50	72.0	a	54.5	b	75.5	a–c	0	g
MW 500 W	30	53.0	c	5.5	d–f	77.0	a–c	1.0	g
	35	35.5	d	11.0	d	81.5	ab	5.5	f
	40	35.5	d	8.0	de	69.5	c	11.0	ef
	45	30.0	d–f	4.0	e–g	66.5	cd	10.5	ef
	50	22.5	e–g	8.0	de	50.0	e	7.5	f
MW 650 W	30	32.0	de	5.5	d–f	76.5	a–c	9.0	ef
	35	23.5	ef	4.0	e–g	52.5	e	24.5	d
	40	22.0	fg	7.5	de	56.0	de	25.0	d
	45	20.5	fg	1.5	gh	35.0	f	40.0	c
	50	1.5	hi	1.0	gh	3.0	i	86.0	a
MW 750 W	30	23.0	ef	3.5	e–g	72.5	bc	13.5	e
	35	13.5	g	2.0	f–h	25.0	g	48.0	c
	40	4.0	h	3.0	e–g	13.0	h	71.0	b
	45	4.5	hi	3.0	e–g	14.0	h	71.5	b
	50	1.0	i	0	h	2.5	i	89.0	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500 W, MW 650 W, MW 750 W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level α = 0.05, according to Duncan's multiple range test.

Untreated seeds in sample IV were characterized by relatively low seed infestation with *A. alternata* and *A. zinniae*—41.0 and 19%, respectively—and very high (95.0%) seed contamination with *Fusarium* spp. (Table 9). Soaking seeds in distilled water generally did not affect the presence of these fungi. An increase in infestation with *A. zinniae* was observed only in the case of seeds soaked in water for 50 s. Treating seeds with fungicide as well as exposing them to microwave radiation at a power output level of 500 W for 35 and 50 s and at power output levels of 650 and 750 W for 30–50 s significantly decreased the percentage of seeds infested with *A. alternata*. A lower percentage of seed infestation with *A. zinniae* was found only after fungicide treatment and in seeds exposed to microwave radiation at power output levels of 650 and 750 W for 45 and 60 s. A decrease in seed infestation with *Fusarium* spp. was recorded after fungicide treatment and in seeds treated with microwave radiation at 500 W for 50 s, at 650 W for 35–50 s and at 750 W for 30–50 s. Treatment with fungicide, as well as with microwave radiation at power output levels of 500 W for 50 s, 650 W for 40–50 s and 750 W for 30–50 s, also resulted in a significant increase in the percentage of seeds free of fungi.

Table 9. The effect of microwave treatment on the health of zinnia seeds—sample IV.

Treatment	Treatment Time (s)	Percent of Seeds Infested with						Seeds Free of Fungi (%)	
		Alternaria alternata		*Alternaria zinniae*		*Fusarium* spp.			
Control [1]	-	41.0	ab [2]	19.0	b–e	95.5	a–c	0	h
Fungicide	-	10.5	d–f	10.0	f–h	74.0	fg	3.0	fg
Distilled water	30	48.0	a	19.5	b–e	98.0	a	0	h
	35	40.5	ab	19.5	b–e	91.0	cd	0	h
	40	52.5	a	22.0	a–d	92.5	b–d	0	h
	45	48.5	a	26.0	a–c	94.5	a–c	0	h
	50	47.5	a	31.5	a	97.5	ab	0	h
MW 500 W	30	42.5	ab	29.0	ab	91.0	cd	0	h
	35	19.0	c	25.0	a–c	95.5	a–c	0	h
	40	35.0	b	28.0	ab	91.5	cd	0	h
	45	33.5	b	29.0	ab	91.5	b–d	0	h
	50	11.5	c–e	16.5	c–f	75.5	fg	7.5	f
MW 650 W	30	15.5	cd	18.0	b–e	91.0	cd	1.5	gh
	35	18.0	cd	18.5	b–e	87.5	de	1.0	gh
	40	20.0	c	18.5	b–e	80.5	ef	4.0	f
	45	12.0	c–e	8.0	gh	61.0	h	21.5	d
	50	1.5	g	4.5	hi	15.9	j	70.0	b
MW 750 W	30	12.5	cd	24.5	a–c	67.5	gh	13.0	e
	35	5.5	ef	12.0	e–g	40.0	i	40.0	c
	40	5.5	f	13.5	d–g	48.5	i	42.0	c
	45	0.5	g	7.0	gh	12.0	jk	69.0	b
	50	1.0	g	2.0	i	7.5	k	79.0	a

[1] Control—untreated seeds; Fungicide—seeds treated with fungicide Zaprawa Nasienna T 75 DS/WS at a dose of 5g kg^{-1} seeds; distilled water—seeds soaked in distilled water; MW 500 W, MW 650 W, MW 750 W—seeds treated with microwaves at power output of 500, 650 and 750 W, respectively. [2] Means in columns followed by the same letter are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

4. Discussion

Tested samples varied significantly in seed germination and infestation with fungi as well as in their response to microwave treatment; nevertheless, fungi from genera *Alternaria* and *Fusarium* were dominant species in all samples. Samples I and II were characterized by higher initial seed germination (54.7 and 44.7%, respectively) than samples III and IV (32.3 and 31.3%, respectively); on the other hand, seeds in samples II and III showed much higher incidences of *A. zinniae* (77.5 and 71.5% respectively) than seeds in samples I and IV (26.5 and 19.0%, respectively). Complete control of *A. zinniae* was achieved only for sample III at the highest power output and longest exposure time tested. However, several experimental variants (power output, treatment time) significantly reduced seed infestation with this pathogen, as well as with *A. alternata* and *Fusarium* spp., in all samples. This was related to a significant increase in the percentage of seeds free of fungi and in many cases with a decrease in the percentage of abnormal diseased seedlings. Moreover, after numerous microwave treatments, improvement in seed germination was observed in samples I, III and IV. Only sample II was characterized by high infestation with *A. zinniae*; none of the treatments significantly increased seed germination at the first and final counts. Referring to *A. zinniae*, the difference in response to treatment was especially visible between samples II and III. In the case of sample II, applied treatments' variants decreased the percentage of seeds infested with this fungus from 77.5% in control to

17.0–56.5%, depending on microwave power output and treatment time, while in sample III the reduction was much more significant, from 71.5% in untreated seeds to 0–11% after treatment. It was probably caused by the superficial location of this fungus on sample III seeds. External location may also explain why soaking seeds in water in most cases reduced seed infestation with this pathogen to some extent. According to Maude [34], fungi of the genus *Alternaria* are usually associated with the seed surface or the outer layers of seeds. However, our previous research has shown [35] that both *Alternaria* spp. and *Fusarium* spp. frequently also invade the deeper tissues of zinnia seeds, including the seed embryo; thus, their total eradication by thermotherapy may not be possible without jeopardizing seed viability. In this case, it has to be taken into consideration that the "safe" microwave treatment will only reduce the presence of pathogenic *Alternaria* species on the surface and in the external tissues of the seeds. Therefore, supplementary treatment may be necessary to provide effective control of the pathogens located in the deeper parts of the seed. It seems crucial for the final plant stand, because deep-seated *A. zinniae* seed infection may lead to the pre-emergent death of seedlings [36]. Attempts to combine microwave seed treatment with other methods of pathogen control have already been made. For example, Friesen et al. [37] applied microwave treatment as an inexpensive addition to chemical seed treatment against *Colletotrichum lindemuthianum* (anthracnose) in bean. The use of synthetic chemicals in organic farming is banned; hence, other physical or biological methods should be investigated to support microwave treatment in this type of plant production.

Tylkowska et al. [22] and Cavalcante and Muchovej [19] reported that multi-celled spores, such as *Alternaria* spp. and *Fusarium* spp., as well as thick-walled and dark spores, such as *Alternaria* spp., are less susceptible to microwave irradiation than one-celled and hyaline spores. Therefore, to control these fungi a higher microwave power output and longer exposition time seem to be necessary. However, the increase in temperature may not only cause the denaturation of proteins, enzymes and nucleic acids in microorganisms exposed to microwave radiation but can also negatively affect the physiological balance and function of seed cells [14–16]. It has been reported that the severity of DNA injury increased with rising temperatures [36]. In the present experiment, the deterioration of seed germination was observed only when seeds in samples I and II were exposed to microwave radiation at power outputs of 650 and 750 W for 50 s, and when seeds of sample III and IV were treated at 750 W power output for the same time, i.e., when water temperature exceeded 60 °C. It can be concluded that an increase in water temperature during microwave treatment over this limit can adversely affect zinnia seed germination, especially if initial seed quality is poor. This is in agreement with Aladjadjiyan's [38] suggestion that greater energy absorbed by molecules at a higher power output level and longer exposure time could have a lethal effect on cell functions. Although a thermal effect induced by microwave irradiation seems to be the most important factor influencing seed associated microorganisms and the seed itself, non-thermal effects of microwaves, including several physiological and biochemical changes, also have to be taken into consideration, as they can modify the pathogenicity of microorganisms [15].

It has been reported that with optimized treatment conditions, microwave irradiation has the ability to inhibit the growth of seedborne microorganisms without seed viability deterioration [15,39]. Nevertheless, maintaining these optimal conditions during treatment could be a challenge, especially if a standard microwave oven is used. In the present experiment, the influence of treatment duration on seed germination and health was uneven in some cases. It was probably caused by variation in water temperature between replications for each treatment variants as a result of the lack of homogeneity of the field distribution in the microwave oven. In the oven, microwaves resonate in the cavity and form standing waves, and the nodes and antinodes of these waves can cause products to burn in some places and remain cool in others [9]. This problem of inconsistency in microwave treatment results was also reported by Friesen et al. [37]. For a larger-scale application, maintaining constant temperature during microwave treatment seems to be even more challenging than in laboratory conditions and requires further investigation.

5. Conclusions

The results of the present study showed that the microwave irradiation of zinnia seeds immersed in water may significantly reduce their infestation with fungi without negative effects on germination, although the effectiveness of the treatment is strongly related to initial seed quality and presumably the location of seed-associated microorganisms. Moreover, to avoid the deterioration of zinnia seed germination during microwave treatment, water temperature, despite the microwaves' output power and treatment time, should not exceed 60 °C.

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