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The Effect of Microwave Treatment on Germination and Health of Carrot (*Daucus carota* L.) Seeds

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Abstract: The aim of the study was to evaluate the effect of microwave treatment on seed germination and health of carrot seeds using two seed lots naturally infected with the pathogens *Alternaria dauci* and *A. radicina*. Seeds of cv. Amsterdam and cv. Berlikumer varied in seed germination at the final count (50% and 29%, respectively), and seed infestation with *A. radicina* (38% and 5%, respectively). For treatment, seeds were placed in a Petri dish (dry treatment) or in a beaker with distilled water (wet treatment) and irradiated at power output levels 500, 650 and 750 W for 15, 30, 45, 60, 75, and 90 s. Germination and health were determined in treated and untreated (control) seed samples. Wet treatment controlled seed-borne fungi more efficiently than dry treatment. However, the exposure duration longer than 60 s frequently resulted in deterioration of seed germination. The highest seed germination in cv. Amsterdam was observed after microwave wet treatment at power output levels of 500 W for 75 s (81%), 650 W for 45 s (85%), and 750 W for 60 s (77%), whereas in the case of cv. Berlikumer this occurred when wet seeds were treated at 500 and 650 W for 60 s (46% and 43% respectively). Treating seeds soaked in water with microwaves for a period longer than 30 s, regardless of the power output, significantly decreased seed infestation with *Alternaria* spp. in both samples.

Keywords: *Alternaria alternata*; *Alternaria dauci*; *Alternaria radicina*; carrot seeds; germination; microwave treatment

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1. Introduction

Synthetic fungicides are commonly applied in conventional agriculture for the control of seed-borne fungi. In the last few decades, however, due to the development of organic farming, various methods of physical seed treatment are acquiring increasing interest. These methods are mainly based on the use of thermal energy in a dry (e.g., elevated temperatures, solar heat) or wet (e.g., hot water, aerated steam) form. However, radiation (e.g., solar, ionizing, microwave) is also being increasingly applied for seed treatment [1]. Microwaves are ultra-high frequency electromagnetic waves widely used in many areas of human life, such as the agri-food industry, communication, medicine, and metallurgy. Thermal effects of microwave treatment are the best known, although, considering the influence of microwaves on living organisms, some nonthermal effects, leading to various types of molecular transformations and alterations, have been also suggested [2]. Microwave treatment was efficiently used for the control of several seed-borne fungi, such as *Ascochyta lentis* and *Botrytis cinerea* in lentil [3,4], *Fusarium graminearum* in wheat [5], *Colletotrichum lindemuthianum* in bean [6], *Fusarium* spp. and *Microdochium nivale* in wheat [7], and *Alternaria* spp. and *Ustilago nuda* in barley [8,9]. Biological effects of microwaves depend on the field strength and frequency, wave forms, and duration of exposure [10]. The prolongation of microwave treatment and an increase in the power output and frequency of waves, associated with an increase in temperature, usually results in a deterioration in seed viability [5,6,11–16]. In contrast, it has been reported that properly applied microwave treatment can significantly improve seed germination and seedling emergence [10,15–20].

Carrot (*Daucus carota* L.) is among the most popular and economically important vegetables in the world [21]. One of the main worldwide problems associated with the production of this crop are diseases caused by seed-transmitted fungi from the genus *Alternaria*, i.e., *A. dauci*, which is responsible for leaf blight, and *A. radicina*, which is a causal agent of black rot of roots and seedling damping-off [22]. *Alternaria alternata*, another fungus from this genus commonly identified on carrot seeds, has been reported by some researchers as a weak pathogen of this plant. The abundant presence of *A. alternata*, especially in connection with seed infestation with *A. dauci* and/or *A. radicina*, may negatively affect seed germination [23]. The widespread nature of *Alternaria* spp. triggers resistance of these fungi to many standard fungicides [24,25]. Moreover, the use of pesticides in organic farming is strictly limited. Thermotherapy, based on hot water treatment, has already been proven to be efficient for the control of *A. dauci* and *A. radicina* on carrot seeds [26]. Microwave radiation in comparison with a conventional heat treatment is simple, rapid, and easy to control. Soaking seeds in water during their exposure to microwaves makes this procedure more complicated, but may protect the seeds from injuries caused by overheating. Therefore, the aim of the present experiment was to investigate the effects of dry and wet microwave treatments on germination and health of carrot seeds.

2. Materials and Methods

Two commercially available standard carrot seed samples (sample I—cv. Amsterdam, lot No PL 004/63/51/020A and sample II—cv. Berlikumer, lot No PL 104/63/51/233A) obtained from Torseed Seed Company in Toruń were tested. The samples varied in seed quality. Both samples were characterized by a low germination at the final count, 50% and 29% in samples I and II, respectively. All seeds of both samples were infested with *Alternaria alternat*, but in sample I a higher percentage (38%) of seed infested with pathogenic *Alternaria radicina* was noted than in sample II (5%). The seeds were treated in a Whirlpool microwave oven type M593 (microwave frequency 2.45 GHz, wave length 12.24 cm).

For each treatment, 1 g of seeds was placed as a single layer in a 9 cm diameter glass Petri dish, or 2 g of seeds was placed in a beaker with a capacity of 100 mL filled with distilled water to a volume of 50 mL. Plates with dry seeds (dry treatment) and beakers with seeds soaked in distilled water (wet treatment) were placed centrally in the microwave oven and treated at three different power output levels—500, 650, and 750 W—for 15, 30, 45, 60, 75, and 90 s. The control for both dry and wet treatments included untreated seeds. Directly after wet treatment, seeds were transferred into a sieve and cooled under tap water for 2 min and then dried for 24 h at 20 °C and 45% RH. In addition, after each wet treatment, the water temperature was measured. The moisture content of dry untreated and dry treated seeds was evaluated using the high-constant-temperature oven method, based on the International Seed Testing Association (ISTA) Rules [25]. The test was carried out in two replicates of 0.5 g of seeds from each treatment. The seeds were dried at 130 °C for 1 h and the moisture content was calculated based on the difference between seed weight before and after drying.

For untreated and microwave treated seeds, germination and health tests were performed. Seed germination was evaluated on six replicates of 50 seeds from each treatment. Seeds were placed in 9 cm diameter Petri dishes (50 seeds per dish) on six layers of blotter paper moistened with distilled water and then incubated for 14 days at 20 °C in the dark. After 7 and 14 days of incubation, germination at the first and final counts (the percentage of normal seedlings) was evaluated, respectively. Moreover, after 14 days the percentages of abnormal diseased seedlings, abnormal deformed seedlings, and ungerminated fresh and dead seeds were determined according to ISTA Rules [27].

The deep-freeze-blotter test was applied for seed health analysis [28,29]. For each treatment, 200 seeds, i.e., 5 replicates of 40 seeds, were tested. The seeds were placed in 9 cm diameter Petri dishes on six layers of blotter paper moistened with distilled water, 20 seeds per dish. The seeds were incubated in darkness at 20 °C for 3 days, at −20 °C for 24 h, and then for 8 days at 20 °C under 12 h alternating cycles of NUV light and darkness. Then, the fungi were identified on the basis of their growth and sporulation using stereoscopic and compound microscopes [30,31]. The percentages of seeds infested with individual fungi and seeds free of fungi were determined.

All parameters describing seed germination and the percentages of seed infestation with individual 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. Effect of Microwave Treatment on Carrot Seed Germination

In the case of sample I, dry treatment for 45 and 75 s with microwave radiation at power output of 500 W, for 75 s at power output of 650 W, and for 30 s at power output of 750 W resulted in a significant increase in seed germination at the first count compared to the control (Table 1). The improvement in this parameter was also observed if seeds soaked in water were exposed to microwave radiation at 500 W for 15, 45, 60 and 75 s, at 650 W for 15, 30, 45 and 60 s, and at 750 W for 15, 45 and 60 s. In contrast, a decrease in germination at the first count was noted for dry seeds treated with microwave radiation at power output levels of 500 and 650 W for 30 s, and at power output of 750 W for 15 and 90 s, and in the case of seeds soaked in distilled water exposed to microwave radiation at 750 W for 30 s, at 650 and 750 W for 75 s, and at 500, 650 and 750 W for 90 s.

Exposure of seeds soaked in distilled water to microwave radiation at power output of 500 W for 15, 45, 60 and 75 s, at power output of 650 W for 15, 30, 45 and 60 s, and at power output of 750 W for 15, 45 and 60 s positively affected seed germination at the final count. Dry seeds treated for 45 and 75 s with microwave radiation at 500 W and for 75 s at 650 W were also characterized by significantly higher germination at the final count than untreated seeds. On the contrary, the deterioration of this parameter was observed for dry seeds exposed to microwave radiation at a power output level of 750 W for 15 and 90 s, and in the case of wet seeds treated with microwave radiation at 650 W for 75 and 90 s, and at 750 W for 30, 75 and 90 s.

The percentage of abnormal diseased seedlings decreased significantly after the exposure of seeds soaked in distilled water to microwave radiation at power output levels of 500, 650, and 750 W for 15, 45, 60, 75 and 90 s, and after treating dry seeds with microwave radiation at 500 W for 45 s, at 650 W for 75 s, and at 750 W for 90 s (Table 1). The increase in the percentage of these seedlings was observed only in the case of wet seeds exposed for 30 s to microwave radiation at power output of 750 W.

The percentage of abnormal deformed seedlings in all treatments was generally low and ranged from 0 to 11.3% (Table 1).

Exposure of dry seeds to microwave radiation of 750 W for 15 and 90 s resulted in a significant increase in the percentage of dead seeds (Table 1). By comparison, a reduction in the number of these seeds was noted after dry treatment with microwave radiation at power output levels of 500 W for 75 s and 750 W for 30 s, and in the case of seeds soaked in water and exposed to microwave radiation at 500 W for 15, 45, 60, 75 and 90 s, at 650 W for 15, 30, 45, 60 and 75 s, and at 750 W for 30, 45 and 60 s.

The control was characterized by a low percentage of fresh seeds (Table 1). Dry treatment in most cases did not influence the occurrence of these seeds, whereas the increase in the percentage of fresh seeds was frequently recorded after wet treatment. The highest numbers of these seeds were found after exposure to microwave radiation at power output of 650 and 750 W for 75 and 90 s.

Table 1. The effect of microwave treatment on carrot seed germination—sample I.

Treatment	Power Output (W)	Time (s)	Germination at the First Count (%)		Germination at the Final Count (%)		Abnormal Diseased Seedlings (%)		Abnormal Deformed Seedlings (%)		Dead Seeds (%)		Fresh Seeds (%)	
dry	500	15	58.0	i–l [1]	60.3	h–k	7.0	d–k [1]	29.0	i–m	7.0	d–k [1]	2.3	a–c
		30	37.0	e	42.3	c–e	15.0	mn	39.3	m	15.0	mn	2.3	a–d
		45	67.0	lm	67.3	k–p	8.3	e–m	20.0	f–i	8.3	e–m	2.3	a–c
		60	42.3	e–h	50.0	e–h	11.0	i–n	36.0	lm	11.0	i–n	2.3	a–d
		75	62.0	kl	63.0	i–m	5.0	d–g	27.0	h–l	5.0	d–g	4.3	a–e
		90	44.0	e–h	50.0	e–h	12.0	k–n	33.0	lm	12.0	k–n	3.0	a–d
	650	15	57.0	i–l	59.0	h–l	8.0	e–l	27.0	h–l	8.0	e–l	4.0	a–f
		30	38.0	ef	43.3	d–f	11.0	h–n	40.3	m	11.0	h–n	2.3	a–c
		45	50.0	h–j	54.0	f–j	10.0	g–m	31.3	k–m	10.0	g–m	1.7	ab
		60	52.0	h–k	54.0	g–j	8.0	e–l	32.0	k–m	8.0	e–l	5.0	bf
		75	64.0	l	65.0	k–n	8.0	e–l	20.0	f–i	8.0	e–l	4.0	a–f
		90	39.0	e–g	45.3	d–g	14.0	l–n	33.0	lm	14.0	l–n	5.7	bf
	750	15	27.0	d	33.0	bc	30.0	o	25.0	h–l	30.0	o	6.7	d–g
		30	61.0	kl	61.0	h–l	5.3	d–h	30.3	j–m	5.3	d–h	3.3	a–e
		45	48.0	g–i	51.0	e–h	6.0	d–j	36.0	lm	6.0	d–j	5.7	c–f
		60	57.0	i–l	59.0	h–l	8.0	e–l	30.0	i–m	8.0	e–l	2.0	a–c
		75	57.3	i–l	59.0	h–l	7.0	d–k	30.3	j–m	7.0	d–k	2.7	a–d
		90	17.3	c	27.0	b	40.0	p	15.0	e–g	40.0	p	14.0	h
wet	500	15	67.0	lm	67.0	k–o	5.0	c–f	21.0	f–j	5.0	c–f	6.0	d–g
		30	47.0	f–i	53.0	e–i	7.0	d–k	35.0	lm	7.0	d–k	3.0	a–e
		45	63.0	l	71.0	m–p	6.0	d–i	17.3	f–h	6.0	d–i	4.7	b–f
		60	61.0	kl	74.0	n–q	2.0	a–c	15.0	e–g	2.0	a–c	6.0	d–g
		75	61.0	kl	81.0	qr	1.0	a	5.0	b	1.0	a	10.0	gh
		90	9.0	b	51.0	e–h	3.0	b–d	6.3	bc	3.0	b–d	28.3	i
	650	15	59.0	j–l	69.0	l–p	5.0	c–e	19.3	f–i	5.0	c–e	3.7	a–e
		30	61.0	kl	64.0	j–m	5.3	c–f	26.0	h–l	5.3	c–f	2.3	a–d
		45	76.0	n	85.0	r	1.0	a	5.0	bc	1.0	a	7.0	e–g
		60	67.0	lm	76.3	o–q	1.0	a	10.3	c–e	1.0	a	9.3	f–h
		75	0	a	1.0	a	1.3	ab	0.3	a	1.3	ab	95.3	l
		90	1.0	a	1.0	a	14.3	n	0.3	a	14.3	n	84.3	j
	750	15	65.3	l–m	68.0	k–p	6.0	d–j	22.0	g–k	6.0	d–j	3.0	a–e
		30	21.0	cd	39.0	cd	5.3	b–e	51.0	n	5.3	b–e	3.7	a–e
		45	73.3	mn	74.0	n–q	4.0	b–d	12.3	d–f	4.0	b–d	5.7	c–g
		60	62.3	kl	77.0	pq	1.0	a	9.0	b–d	1.0	a	11.0	gh
		75	0	a	0.3	a	7.0	d–k	0	a	7.0	d–k	90.7	k
		90	0	a	0	a	9.0	f–n	0	a	9.0	f–n	91.0	k
Control—untreated seeds			48.3	g–i	50.0	e–h	36.3	lm	1	a–c	11.0	j–n	1.7	a

[1] Means in columns followed by the same letter are not significantly different at the level α = 0.05, according to Duncan's multiple range test.

In the case of sample II, the increase in seed germination at the first count, compared to the control, was observed only after wet treatment when seeds were exposed for 60 s to microwave radiation at a power output level of 500 W (Table 2). By comparison, exposure of dry seeds to microwave radiation at 500 W for 15, 30, 75 and 90 s, and at 650 W for 45, 75 and 90 s, in addition to wet treatment with microwave radiation at power output levels of 650 W for 45, 75 and 90 s, and 750 W for 30, 60, 75 and 90 s, deteriorated this parameter significantly.

Table 2. The effect of microwave treatment on carrot seed germination—sample II.

Treatment	Power Output (W)	Time (s)	Germination at the First Count (%)		Germination at the Final Count (%)		Abnormal Diseased Seedlings (%)		Abnormal Deformed Seedlings (%)		Dead Seeds (%)		Fresh Seeds (%)	
dry	500	15	14.3	d–f [1]	21.0	d–g	50.3	i–k	6.0	c–h	17.0	d–g [1]	5.7	c–h
		30	6.0	bc	8.3	b	13.3	b	2.0	a–d	52.0	l	24.3	lm
		45	21.3	f–i	28.3	f–h	49.0	i–k	4.0	b–g	14.0	c–f	4.7	c–g
		60	23.0	f–i	30.0	gh	35.0	f–h	5.0	b–g	23.0	f–i	7.0	d–i
		75	11.3	de	17.3	c–e	40.0	f–i	3.0	b–f	31.3	ij	8.3	d–j
		90	6.0	bc	12.0	bc	12.3	b	5.0	b–g	60.0	l	10.7	h–k
	650	15	19.0	f–h	32.0	g–i	44.0	h–j	3.0	b–f	18.0	d–g	3.0	a–e
		30	27.0	hi	32.0	g–i	47.0	h–k	3.0	b–f	17.0	d–f	1.0	a
		45	11.0	cd	25.0	e–h	31.0	e–g	6.0	c–h	32.3	jk	5.7	c–g
		60	31.0	ij	35.0	h–j	43.0	h–j	3.0	b–f	16.3	d–f	2.7	a–c
		75	0.3	a	8.3	b	14.3	b	7.0	f–h	56.0	l	14.3	jk
		90	11.3	de	22.0	d–g	24.0	c–e	5.0	b–g	41.3	k	7.7	d–j
	750	15	24.0	g–i	27.0	f–h	52.0	i–k	2.3	b–e	15.0	d–f	3.7	a–d
		30	21.3	f–i	25.0	e–h	51.0	i–k	3.3	b–f	19.0	d–h	1.7	ab
		45	22.0	f–i	29.0	f–h	44.0	h–j	4.0	b–g	19.0	d–g	4.0	a–e
		60	27.0	hi	30.0	gh	44.3	h–j	3.3	b–f	20.0	e–h	2.3	a–c
		75	25.0	hi	30.0	gh	39.0	f–i	4.0	b–g	22.0	f–i	5.0	b–g
		90	28.0	hi	30.0	gh	45.0	h–j	4.0	b–f	16.3	d–g	4.7	b–g
wet	500	15	26.0	hi	30.3	gh	49.0	i–k	4.0	b–f	13.0	c–e	3.7	a–f
		30	27.3	hi	31.0	gh	44.0	h–j	3.0	a–d	17.0	d–g	5.0	c–h
		45	30.3	ij	35.0	h–j	45.0	h–j	3.3	b–f	9.0	b	7.7	d–i
		60	38.0	j	46.0	J	31.0	e–g	2.0	a–c	11.0	b–d	10.0	g–j
		75	19.0	e–h	37.0	h–j	21.3	b–d	15.0	ij	1.0	a	25.7	m
		90	22.0	f–i	44.3	J	19.0	bc	11.0	hi	9.0	bc	16.7	kl
	650	15	28.0	hi	31.0	gh	47.0	h–k	2.0	a–d	15.0	c–f	5.0	a–e
		30	30.0	ij	37.0	h–j	41.0	f–i	2.0	a–d	18.0	d–g	2.0	a–c
		45	16.0	d–g	19.0	d–f	59.0	k	6.0	e–h	6.3	b	9.7	f–j
		60	31.0	ij	43.0	Ij	31.0	d–f	5.3	d–h	8.0	bc	12.7	i–k
		75	0.3	a	2.0	A	0	a	20.3	j	7.0	b	70.7	n
		90	0	a	0	A	0	a	2.0	a–d	28.3	h–j	69.7	n
	750	15	23.0	g–i	27.3	f–h	48.0	i–k	4.3	b–g	15.3	d–f	5.0	b–g
		30	7.0	b	22.0	d–g	55.3	jk	2.0	ab	13.0	c–e	7.7	d–i
		45	30.0	ij	36.0	h–j	42.3	g–i	6.0	e–h	6.3	b	9.3	e–j
		60	13.0	de	16.0	Cd	52.0	i–k	3.3	b–g	2.0	a	26.7	m
		75	1.3	a	2.0	A	2.0	a	8.3	g–i	19.3	d–h	68.3	n
		90	0	a	0.3	A	0.3	a	0	a	17.3	d–g	82.0	o
Control—untreated seeds			26.0	hi	29.0	f–h	39.3	f–i	3.3	b–g	26.0	g–j	2.3	a

[1] Means in columns followed by the same letter are not significantly different at the level α = 0.05, according to Duncan's multiple range test.

Treating seeds soaked in distilled water with microwave radiation at 500 W for 60 and 90 s and at 650 W for 60 s resulted in a significant improvement in seed germination at the final count. However, exposure of dry seeds to microwave radiation at 500 W for 30, 75 and 90 s, and at 650 W for 75 s, in addition to treating seeds soaked in water with microwave radiation at power output levels of 650 W for 75 and 90 s, and 750 W for 60, 65 and 90 s, deteriorated this parameter.

Wet treatment with microwave radiation at power output levels of 650 and 750 W for 45 and 30 s, respectively, resulted in an increase in the percentage of abnormal diseased seedlings (Table 2). Nevertheless, treating seeds soaked in water with microwave radiation for 75 and 90 s, regardless of the applied power output, and exposure of dry seeds to microwave radiation at 500 W for 30 and 90 s, and at 650 W for 75 and 90 s, significantly reduced the number of these seedlings.

The percentage of abnormal deformed seedlings in all treatments was generally low (Table 2). An increase in the number of these seedlings was noted only after wet treatment, for seeds exposed to microwave radiation at power output of 500 W for 75 and 90 s and 650 W for 75 s.

A significant increase in the percentage of dead seeds was recorded after exposure of dry seeds to microwave radiation at power output levels of 500 W for 30 and 90 s, and 650 W for 75 and 90 s (Table 2). In contrast, a decrease in this parameter was observed if dry seeds were treated with microwave radiation at 500 W for 45 s, at 650 W for 30 and 60 s, and at 750 W for 15 s, and in the case of wet seeds exposed to microwave radiation at 500 W for 15, 45, 60, 75 and 90 s, at 650 W for 15, 45, 60 and 75 s, and at 750 W for periods shorter than 75 s.

The percentage of fresh seeds in the control was very low (Table 2). Most of the applied treatment variants, both dry and wet, resulted in an increase in the number of these seeds. The highest percentages of fresh seeds were found after exposure to microwave radiation at power output of 650 and 750 W for 75 and 90 s.

3.2. Effect of Microwave Treatment on Carrot Seeds Infestation with Fungi

The numerous fungi were identified on tested carrot seeds, i.e., *Acremoniella atra* (Corda) Sacc., *Alternaria alternata* (Fr.) Keissler, *A. dauci* (Kühn) Groveset Skolko, *A. radicina* Meier, Drechsler et Eddy, *Bipolaris* sp., *Colletotrichum* spp., *Cladosporium* spp., *Epicoccum nigrum* Link, *Fusarium* spp., *Melanospora simplex* Corda, *Papulaspora* sp., *Penicillium* spp., *Phoma sp.*, *Rhizopus stolonifer* (Ehrenb.) Vuill., *Stemphylium botryosum* Wallr., *Trichothecium* sp., *Ulocladium* spp. and *Verticillium* spp. Among these, fungi of the genus *Alternaria* prevailed. *Alternaria alternata* was recorded on all untreated seeds of both samples. *Alternaria dauci* infested 4.5% seeds of sample I and 5.0% seeds of sample II. The percentage of seeds infested with *A. radicina* in sample I was 38.0%, whereas in sample II it was only 5.0% (Tables 3 and 4).

Generally, wet treatment more efficiently controlled *Alternaria* spp. on seeds of both samples than dry treatment. Exposure of sample I seeds soaked in water to microwave radiation at power output levels of 500 W for 30, 45, 50, 75 and 90 s, 650 W for 45, 60, 75 and 90 s, and 750 W for 15, 30, 45, 60, 75 and 90 s resulted in a significant decrease in the percentage of seeds infested with *A. alternata*. On the contrary, none of applied dry treatment variants affected the presence of this fungus (Table 3).

In sample II, the decrease in seed infestation with *A. radicina* was observed only when seeds soaked in water were exposed to microwave radiation at 500 and 750 W for 45, 60, 75 and 90 s, and at 650 W for 15, 45, 60, 75 and 90 s (Table 4).

In both samples, the significant reduction in seed infestation with fungi after wet treatment resulted in the significant increase in the percentage of seeds free of fungi (Tables 3 and 4). This phenomenon was observed especially when seeds soaked in water were exposed to microwave radiation for a period longer than 30 s, regardless of the power output applied. However, in some cases, 30 s treatment also caused a small increase in the number of these seeds. By comparison, after dry treatment, seeds free of fungi were generally not noted, and a significant increase in the percentage of these seeds was observed only in sample II, when seeds were exposed to microwave radiation at power output of 650 W for 30 s.

Table 3. Seed infestation with *Alternaria* spp. and the number of seeds free of fungi after microwave treatment of carrot seeds—sample I.

Treatment	Power Output (W)	Time (s)	Seed Infestation with *Alternaria* spp. (%)						Seeds Free of Fungi (%)	
			A. alternata		*A. dauci*		*A. radicina*			
dry	500	15	100.0	h [1]	4.5	d–h	34.5	j–m	0	a
		30	100.0	h	4.5	c–h	28.5	h–l	0	a
		45	100.0	h	6.0	f–h	40.0	m	0	a
		60	100.0	h	6.0	e–h	31.5	i–m	0	a
		75	100.0	h	7.0	gh	37.0	k–m	0	a
		90	100.0	h	9.0	h	27.0	g–j	0	a
	650	15	99.5	h	4.0	c–h	15.5	d–f	0	a
		30	100.0	h	4.5	c–h	31.5	i–m	0	a
		45	99.5	h	4.5	d–h	20.5	e–h	0	a
		60	99.5	h	3.5	b–g	23.0	e–i	0	a
		75	100.0	h	1.5	a–c	25.0	f–j	0	a
		90	100.0	h	3.0	b–h	32.0	i–m	0	a
	750	15	100.0	h	1.5	a–d	23.5	e–i	0	a
		30	100.0	h	3.5	b–g	25.5	g–j	0	a
		45	100.0	h	3.0	b–g	22.0	e–i	0	a
		60	100.0	h	4.5	c–h	28.0	h–l	0	a
		75	100.0	h	7.0	e–h	26.5	h–k	0	a
		90	100.0	h	4.5	d–h	9.5	e–h	0	a
wet	500	15	100.0	h	4.5	c–h	16.5	d–g	0	a
		30	88.0	g	2.0	a–f	15.5	de	6.5	b
		45	82.5	f	5.0	c–h	6.0	c	9.5	b
		60	13.5	cd	0	a	1.0	ab	72.5	d
		75	2.0	b	0	a	0.5	ab	96.0	i
		90	9.5	bc	0	a	0	a	73.0	d
	650	15	100.0	h	3.5	c–h	25.5	g–j	0	a
		30	100.0	h	2.5	b–g	20.0	e–h	0	a
		45	14.0	cd	0.5	ab	1.0	ab	81.5	e
		60	9.0	bc	0	a	1.5	ab	86.5	f
		75	2.5	a	0	a	0	a	89.0	fg
		90	15.5	d	0	a	0	a	81.0	e
	750	15	100.0	h	1.0	a–c	27.0	h–k	0	a
		30	85.0	fg	1.5	a–e	9.5	cd	7.5	b
		45	29.5	e	0.5	ab	2.0	ab	66.5	e
		60	11.0	cd	0	a	2.0	b	81.5	c
		75	1.5	a	0	a	0	a	95.0	hi
		90	1.0	a	0	a	0	a	92.0	gh
Control—untreated seeds			100.0	h	4.5	c–h	38.0	lm	0	a

[1] Means in columns followed by the same letter are not significantly different at the level $\alpha = 0.05$, according to Duncan's multiple range test.

Table 4. Seed infestation with *Alternaria* spp. and the number of seeds free of fungi after microwave treatment of carrot seeds—sample II.

Treatment	Power Output (W)	Time (s)	Seed infestation with *Alternaria* spp. (%)						Seeds Free of Fungi (%)	
			A. alternata		*A. dauci*		*A. radicina*			
dry	500	15	100.0	k [1]	5.0	d	3.5	b–g	0	a
		30	98.5	h–k	2.0	a–d	3.0	c–g	0	a
		45	99.0	i–k	5.5	d	4.5	d–g	0	a
		60	97.5	h–k	2.0	b–d	2.0	b–f	0	a
		75	99.5	jk	4.5	cd	2.5	b–e	0	a
		90	97.5	h–k	4.0	cd	4.0	d–g	0	a
	650	15	100.0	i–k	3.5	cd	2.5	b–e	0	a
		30	80.0	fg	2.5	b–d	5.5	e–g	9.0	b
		45	98.0	h–k	2.5	b–d	5.0	e–g	0.5	a
		60	98.0	h–k	5.0	d	7.0	fg	0	a
		75	98.0	h–k	3.5	cd	4.5	d–g	0	a
		90	97.0	h–k	4.0	b–d	4.0	d–g	1.0	a
	750	15	98.5	h–k	4.0	cd	6.5	fg	0	a
		30	99.0	i–k	5.0	d	3.5	d–g	0	a
		45	96.0	hi	2.5	b–d	7.5	g	1.5	a
		60	99.5	jk	4.0	b–d	5.0	e–g	0	a
		75	99.0	i–k	4.5	cd	3.5	d–g	0	a
		90	99.0	i–k	5.0	d	5.5	e–g	0	a
wet	500	15	99.5	jk	5.0	d	2.0	a–e	0	a
		30	96.0	h–j	0.5	ab	3.0	c–g	1.0	a
		45	73.0	e	2.0	a–d	1.0	a–d	13.0	c
		60	10.5	c	0	a	1.0	a–d	74.5	e
		75	4.0	ab	0	a	0	a	92.0	f
		90	4.0	b	0	a	0	a	85.0	ef
	650	15	97.0	h–j	6.0	d	1.0	a–c	1.5	a
		30	84.5	f	2.5	a–d	3.0	c–g	6.0	b
		45	46.0	d	1.0	a–c	0	a	45.0	d
		60	2.5	ab	0	a	0	a	91.0	f
		75	0.5	a	0	a	0	a	97.5	g
		90	1.0	ab	0	a	0	a	92.0	f
	750	15	97.5	h–k	1.5	a–d	4.0	e–g	0.5	a
		30	94.5	gh	2.5	a–d	4.0	e–g	2.0	a
		45	41.0	d	1.0	a–c	1.0	a–d	42.0	d
		60	2.0	ab	0	a	0.5	ab	91.0	f
		75	1.5	ab	0	a	0	a	98.5	g
		90	2.5	ab	0	a	0	a	88.0	f
Control—untreated seeds			100.0	k	5.0	d	5.0	e–g	0	a

[1] Means in columns followed by the same letter are not significantly different at the level $\alpha = 0.05$, according to Duncan's multiple range test.

3.3. Effect of Dry Microwave Treatment on Seed Moisture Content

Generally, after dry microwave treatment, seed moisture content decreased in relation to untreated seeds (Table 5). However, the relationship between seed moisture content and treatment time was not linear. The lowest values of this parameter in both samples were observed in the case of seeds treated for 90 s, regardless of the microwave power.

Table 5. The percentage of moisture content in carrot seed after dry microwave treatment.

Power Output (W)	Treatment Time (s)	Moisture Content (%)	
		Sample I	**Sample II**
500	15	7.2	8.7
	30	7.6	8.9
	45	6.6	8.9
	60	6.8	7.8
	75	6.5	8.0
	90	5.0	6.1
650	15	7.0	8.8
	30	6.8	8.1
	45	6.7	9.0
	60	6.6	7.3
	75	5.5	7.0
	90	5.4	6.6
750	15	7.0	8.5
	30	7.8	7.6
	45	6.0	7.9
	60	7.0	7.2
	75	6.4	7.2
	90	4.5	6.2
Control—untreated seeds		8.3	9.6

3.4. Water Temperature after Wet Microwave Treatment

The water temperature after wet treatment rose successively with the increase in the microwave power and exposure time, especially if seeds were treated for longer than 30 s (Table 6).

Table 6. Average water temperature after wet microwave treatment (°C).

Treatment Time (s)	Power Output (W)		
	500	**650**	**750**
15	35.0	39.0	37.5
30	44.0	43.0	47.0
45	48.0	58.0	57.5
60	62.5	63.5	67.0
75	66.0	80.5	87.5
90	74.5	86.0	92.0

4. Discussion

Thermotherapy is one of the oldest approaches of seed sanitation, but because of numerous practical limitations, it has never been widely used in conventional agriculture for the control of seed-borne fungi. The development of organic farming renewed interest in physical seed treatment methods, such as thermotherapy, including the use of hot water, hot humid air, and microwave radiation. Hot humid air (ThermoSeed technology) has been used routinely in Sweden and Norway for many years to control seed-borne pathogens in cereals [32,33]. Microwave technology offers several advantages, including safety, high efficiency, and environmental protection.

The results of the performed experiment showed that wet treatment, i.e., soaking seeds in water during their exposure to microwave radiation, was generally much more effective in controlling the incidence of *Alternaria alternata*, *A. dauci*, and *A. radicina* on carrot seeds in both examined samples than dry treatment. Basically, the dry treatment did not affect the level of seed infestation with *A. alternata* and *A. dauci.* Only in the case of sample I seeds, which were infested with *A. radicina* to a much higher degree than sample

II seeds, some reduction in the incidence of this fungus was found after treating dry seeds with microwave radiation, mainly at power output levels of 650 and 750 W. The moisture content of untreated seeds in sample I was 8.3% and in sample II was 9.6%. Tylkowska et al. [18] reported that treating dry (9.5% m.c.) common bean seeds with microwaves in a microwave oven with a power output of 650 W and frequency of 2450 MHz for 15–120 s did not control *A. alternata* and *Fusarium* spp., but diminished the presence of *Penicillium* spp. It has been suggested that dark, multi-celled, and thick-walled spores, in addition to dark mycelium (e.g., *Alternaria* spp., *Bipolaris* sp.), are less sensitive to microwave radiation than hyaline and one-celled spores (e.g., *Aspergillus* spp., *Penicillium* spp.) [18,34]. The study of Mangwende et al. [35] showed that higher seed moisture content translates to an increase in efficacy of microwave radiation against seed-borne fungi. Water molecules are polar; they rotate when exposed to microwaves and the rotation produces heat. Dried samples are not affected due to the lack of polar molecules, whereas those in the presence of water can reach lethal temperatures [36].

The mechanism of microbial inhibition caused by microwave radiation is based on the internal heating of the seeds resulting from molecular movement in the pulsing electromagnetic field. This leads to the denaturation of proteins, enzymes, and nucleic acids. Proteins are directly damaged by heating as the bonds holding them together are destroyed. However, this also implies the risk of losing enzymatic activities, which are essential for carrying out metabolic processes [36–38]. It has been reported, based on the study performed on *A. parasiticus*, that the severity of DNA injury increased with rising temperatures [37]. Moreover, non-thermal microwave effects in terms of energy required to produce various types of molecular transformation and alterations have been reported [2]. Electroporation is one of the non-thermal effects caused by microwave radiation. Microwaves at sub-lethal temperatures induce the formation of pores in a cellular membrane due to their interaction with polar molecules. These pores allow the cellular contents, including DNA, to leak outside [36]. It has been found that the lethal effect of low-dose microwave radiation (LDMR) on spoilage microorganisms was a result of a disruption of the cell membrane and induced DNA damage [37]. Based on studies concerning the effect of microwave radiation on the death rates of *Escherichia coli,* it has been proposed that microwaves either cause ions to accelerate and collide with other molecules, or cause dipoles to rotate and line up rapidly (2450 million times/s) with an alternating electric field, resulting in a change in the secondary and tertiary structure of proteins of microorganisms [2]. Knox et al. [7], who observed that the number of viable fungal propagules on wheat seeds decreased after microwave treatment, but the level of fungal DNA remained unchanged, concluded that the death of fungi was connected with high temperature and desiccation rather than DNA denaturation. Microwaves cause various biological effects depending upon field strength, frequencies, wave forms, modulation, and duration of exposure [2].

In this study, exposure of dry seeds to microwave radiation generally did not influence their germination or showed adverse effects. An improvement in seed germination at the first and final counts was observed only in sample I, characterized by higher initial seed germination than in sample II, after the use of microwave radiation at power output of 500 W for 45 and 75 s, and 650 W for 75 s. It should be also emphasized that seed germination at the first count is an indicator of seed vigor, because it shows the percentage of normal seedlings that developed in a short time. It has been found that seed moisture content decreased with the increase in the power output and treatment duration. Aladjadjiyan [13] presented the hypothesis that greater energy absorbed by molecules at a higher power output level and longer exposure time may have a deleterious effect on cell functions. The study of Knox et al. [7] revealed that deterioration of wheat seeds' viability as a result of microwave treatment was undeniably linked with a decrease in seed moisture content. Bhaskara Reddy et al. [5] found that germination and vigor of microwave treated wheat seeds was reduced significantly at 8% m.c. According to the authors at this moisture content, the free water was lost sooner than at higher moisture contents (14% and 20%), leaving only the bound water in the seed to absorb energy. During the treatment, progressively lower amounts of microwave power were absorbed by the free water relative to those absorbed by the lipids and proteins of the seeds. At higher moisture content, most of the power is absorbed by the free water in the seed, thus resulting in less damage. Mangwende et al. [35] observed that microwave radiation of moist *Eucalyptus* seeds increased their germination to a larger extent than in the case of dry seeds. Aladjadjiyan [39] also reported that a preliminary soaking of common bean seeds in distilled water increased the effect of microwave stimulation by more than 25%.

It can be supposed that soaking seeds in water might affect their infestation with fungi by removing a superficial inoculum. Our previous experiments showed that soaking carrot seeds in water for a short period of time (15–120 s) did not reduce seed contamination with fungi; on the contrary, the increase in the incidence of some of them was observed (personal observation). However, some fungal propagules present on the seed surface could be removed during rinsing the seeds under tap water after wet microwave treatment.

It was found in the present study that the exposure of seeds soaked in water to microwave radiation, especially for 45–90 s, irrespective of the applied power output level, resulted in a significant decrease in seed infestation with *Alternaria* spp. Moreover, after the wet treatment, high percentages of seeds free of fungi were noted, whereas in the control they were not observed. It is likely that most fungi present on seeds, not only *Alternaria* species, were eradicated by this treatment. From the difference in the efficacy of dry and wet treatments, it may be concluded that *Alternaria* spp. mostly contaminated seeds. Some researchers reported previously that *Alternaria* spp. inoculum is located mainly on the carrot seed surface [25,40,41]. Wet treatment combines the internal heating of seeds caused by microwave radiation with hot water treatment, which mainly affects the fungal inoculum present on the seed surface or located in a few internal layers of a seed. Microwave radiation can rapidly penetrate seeds at the cellular level, killing seed-borne pathogens deeply imbedded in seed tissues. Strandberg and White [42] reported that soaking carrot seeds in hot water at 50 °C for 20 min decreased their infestation with *A. dauci,* whereas Pryor et al. [43] found that hot water treatment at 50 °C for 30 min eliminated *A. radicina.* Hermansen et al. [44] reported that hot water treatment of carrot seeds at 44, 49 and 54 °C generally improved germination of infected seeds and reduced the incidence of *A. dauci*. The treatment at 54 °C for 20 min eradicated this fungus without adversely affecting seed germination, seedling emergence, and yield. According to Nega et al. [26], hot water treatments at 50 °C for 30 min and at 53 °C for 10 min reduced the incidence of fungi from genus *Alternaria* on carrot seeds by 85% to 98%. In our study, the exposure of seeds soaked in water to microwave radiation for 45–120 s, irrespective of the power output level, resulted in a significant decrease in seed infestation with *A. alternata*, *A. dauci*, and *A. radicina*, and in the increase in the percentage of seeds free of fungi in both samples. The average temperature of water after these treatments, depending on the power output and treatment duration, ranged from 48.0 to 92.0 °C. It rose with the increase in the power output level. Unfortunately, the exposure time exceeding 60 s generally had an adverse effect on seed germination, especially at the microwave power output of 650 and 750 W. A significant decrease in seed germination combined with the increase in the percentage of abnormal deformed seedlings was observed in both samples, if water temperature during wet treatment exceeded 80 °C. In sample I, characterized by the higher initial seed vigor and germination, and the much greater infestation with pathogenic *A. radicina* than in sample II, an enhancement of germination at the first and final count was observed in most cases. This was likely caused by the decreased incidence of the pathogen. The most significant improvement in seed germination at the final count was observed when seeds soaked in water were exposed to microwave radiation at 500 W for 75 s, at 650 W for 45 s, and at 750 W for 60 s. The water temperature in these treatments reached 66, 58, and 67 °C, respectively. In sample II, an increase in seed germination was noted after wet treatment at the power output of 500 W for 60 and 90 s (water temperature 62.5 and 74.5 °C, respectively), and 650 W for 60 s (63.5 °C). The water temperature in these treatments was higher than the temperature recommended for hot water treatments mentioned above, but the duration was much shorter. In some cases, the influence of treatment duration on seed germination seems to be inconsistent. Some variation in water temperature between replications for each treatment variants was observed. This was probably caused by the lack of homogeneity of the field distribution in the microwave oven. In the oven, microwaves resonate in the cavity and form standing waves, but the nodes and antinodes of these waves can cause the product to burn in some places but to remain cool in others [45]. Therefore, further experiments are necessary to eliminate this problem during microwave seed treatment.

5. Conclusions

Treating seeds soaked in water with microwave radiation for a period longer than 30 s, regardless of the power output, significantly decreased seed infestation with *Alternaria* spp. in both samples. However, the exposure duration longer than 60 s frequently resulted in deterioration of seed germination.

The results of the present study showed that the wet microwave treatment has the ability to control microbial growth on carrot seeds, but treatment conditions must be optimized to avoid deterioration of seed germination and vigor. Hence, the effects of different factors, such as microwave heating power output, treatment duration, level of seed infestation with microorganisms and their location, structural compounds of pathogens, seed moisture content, and initial seed quality, require further investigation.

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