Article

The Effect of Acetic Acid Treatments on the Quality of Stored Carrot (*Daucus carota* L.) Seeds

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Abstract: *Alternaria dauci* (J.G. Kühn) J.W. Groves et Skolko and *A. radicina* Meier, Drechsler et E.D. Eddy are important seed-transmitted pathogens of carrot. The aim of this study was to evaluate the effects of acetic acid treatments on the quality of stored carrot seeds. Seeds of two samples were soaked for 30 min in 0.5, 1 and 2% acetic acid. Controls included untreated seeds, seeds soaked in distilled water and seeds treated with fungicide Zaprava Nasienna T 75 WS/DS (a.i. thiram 75%). Germination, vigour and health of untreated and treated seeds were evaluated before and after 5 and 12 months of storage at 4 and 20 °C. Seeds of both samples treated with 0.5 and 1% acetic acid were characterized by higher germination capacity after storage than untreated seeds. However, treatments with 1 and 2% acetic acid negatively affected seed vigour. Generally, seeds of both samples treated with acetic acid were characterized by lower infestation with *A. alternata* and *A. radicina* after storage than untreated seeds and seeds soaked in distilled water. Moreover, acetic acid often controlled these fungi more effectively than the fungicide. Regardless of the storage duration, infestation with fungi was higher if seeds of both samples were stored at a lower temperature.

Keywords: acetic acid; *Alternaria* spp.; carrot; seeds; storage

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1. Introduction

Fungi of the genus *Alternaria*, i.e., *A. dauci* (J.G. Kühn) J.W. Groves et Skolko and *A. radicina* Meier, Drechsler et E.D. Eddy, are the most important seed-transmitted pathogens of carrot, reported from many countries all over the world. *Alternaria dauci* is known as a causal agent of leaf blight, and *A. radicina* is mainly responsible for black rot of roots and pre- and post-emergence seedling damping-off [1,2]. *Alternaria alternata* (Fr.) Keissl., another *Alternaria* species commonly associated with seeds of many plants as a saprotroph, is considered by some researchers as a weak pathogen of carrot [1]. The fungicides, such as thiram, chlorothalonil and iprodione, have been used to control *Alternaria* spp. on carrot seeds, however their effectiveness seems to be insufficient in the case of highly infested lots, especially if the pathogen is located in the inner tissues of the seeds [2]. Moreover, the use of synthetic fungicides in organic farming is strictly limited. A good alternative for standard chemical treatment may be in this case application of various compounds of natural origin, such as organic acids. Acetic acid occurs naturally in many fruits as a metabolic intermediate and is one of the main products of lactic acid bacteria (genera in the order *Lactobacilliales*) [3]. The best-known form of acetic acid is vinegar (4–18% acetic acid), commonly used as a food preservative. It is obtained commercially by submerged oxidative fermentation—the process in which acetic acid bacteria (*Acetobacter*) convert ethanol to acetic acid in the presence of oxygen [4]. Acetic acid, because of its well-known antifungal and antibacterial properties, has also been used against plant pathogens, including seed-borne fungi. It has been reported that acetic acid seed treatment successfully reduced common bunt (*Tilletia tritici* (Bjerk.) G. Winter, syn. *T. caries* (DC.) Tul. and C. Tul. and *T. laevis* J.G. Kühn) in wheat [5–7], and leaf stripe (*Pyrenophora graminea* S. Ito and Kurib.)

in spring barley [5], controlled *Fusarium graminearum* Schwabe and *Bipolaris sorokiniana* Shoemaker in barley and wheat seeds [8], and storage mold (*Aspergillus flavus* Link) in canola, corn, rice and wheat seeds [9], decreased zinnia seed infestation with *A. alternata*, *Alternaria zinniae* H. Pape and *Fusarium* spp. [10], and reduced the incidence of *A. alternata*, *A. flavus*, *Aspergillus niger* Tiegh., *Fusarium* spp., *Penicillium* spp., *Rhizoctonia solani* J.G. Kühn, *Rhizopus* sp., *Stemphylium* sp., *Trichothecium* sp. and *Verticillium* sp. on sunflower seeds [11]. In vitro assays, performed by Van der Wolf et al. [12], also exhibited a slight activity of acetic acid against *A. dauci*. Our previous experiments revealed that acetic acid treatment may effectively control *Alternaria* spp. associated with carrot seeds, without a negative effect on seed germination [13]. In practice it may be necessary to store treated seeds for a certain period of time before sowing. Generally, with a few exceptions, treating seeds with fungicides before storage is not recommended because pesticides can harm the seeds, and as a result, reduce their viability. Phytotoxicity can occur after several months of storage, but also after a longer storage period, e.g., 12 months for cereals [14]. Therefore, the question arose whether the organic treatment would affect carrot seed storability? Consequently, the aim of our experiment was to evaluate the possibility of 5- and 12-month storage of carrot seeds treated with acetic acid.

2. Materials and Methods

Two standard carrot seed samples cv. Amsterdam 3 (sample I—lot No PL 009/63/51/020A, sample II—lot No PL 409/63/51/703A), obtained from TORSEED Seed Company in Toruń, were used in the experiment. The samples were selected due to the various levels of seed infestation with fungi. Acetic acid (99.8%) produced by Sigma-Aldrich Co. was applied for seed treatment. Additionally, fungicide Zaprawa Nasienna T 75 WS/DS (a.i. 75% thiram), produced by Organika-Azot in Jaworzno, was used as an alternative chemical control.

Seeds were treated by soaking in 0.5, 1 and 2% acetic acid solutions for 30 min, and next rinsed three times with distilled water. Controls included untreated seeds, seeds soaked in distilled water for 30 min, and seeds treated with Zaprawa Nasienna T 75 WS/DS at a dose of 5 g per kg of seeds. After acetic acid treatments and soaking in distilled water, the seeds were dried at 20 °C and 45% RH for 48 h. Moisture content of dried seeds was evaluated with the high-constant-temperature oven method [15]. Depending on the treatment, the moisture content ranged from 7.2 to 7.7% in sample I and from 7.4 to 7.8% in sample II.

Untreated and treated seeds were stored in plastic, tightly closed containers at 4 and 20 °C. Germination, vigour and health of the seeds were evaluated before and after 5 and 12 months of storage.

Germination and vigour tests were performed separately in six replications of 50 seeds from each treatment. The seeds were incubated in 9 cm diameter Petri dishes on six layers of filter paper, 50 seeds per dish, in darkness at 20 °C. Percentages of normal seedlings (germination capacity), abnormal diseased seedlings and dead seeds were calculated after seven and fourteen days of incubation according to ISTA rules [15]. Mean germination time, characterizing seed vigour, was evaluated based on the number of seeds with visible radicle, counted daily.

The deep-freeze blotter test was performed on five replications of 40 seeds from each treatment according to ISTA rules [16,17]. Seeds were incubated in 9 cm diameter Petri dishes on six layers of filter paper, 20 seeds per dish, for three days at 20 °C in darkness, then transferred to −20 °C for 20 h and subsequently incubated for eight days at 20 °C, under 12 h alternating cycles of NUV light and darkness. After incubation the fungi were identified on the basis of their growth and sporulation using a stereomicroscope and a compound microscope [18,19]. Additionally, the percentage of seeds free of fungi was evaluated.

SeedCalculator version 2.1 software [20] was applied to analyse mean germination time. The results obtained before storage were compared by means of one-way analysis of variance, whereas two-way analysis of variance was used to compare the results obtained after 5 and 12 months of storage. Duncan's multiple range test was applied to estimate the differences between the means at a level $\alpha = 0.05$.

3. Results

The samples varied in terms of seed quality. Sample I was characterized by low seed germination capacity associated with high percentages of abnormal diseased seedlings and dead seeds (Table 1). The improvement of germination capacity was observed only if seeds were treated with fungicide and 0.5% acetic acid solution. All of the applied acetic acid treatments, as well as soaking seeds in distilled water, significantly decreased the number of abnormal diseased seedlings. However, in the case of acetic acid treatments, especially when 2% concentration was used, a significant increase in the number of dead seeds was observed. Sample II showed considerably higher seed germination capacity compared to sample I, which was related to lower numbers of abnormal diseased seedlings and dead seeds. The improvement of germination capacity was observed only if seeds were treated with fungicide and soaked in distilled water. Acetic acid did not affect this parameter, but at 1% concentration increased the number of dead seeds.

Table 1. Effects of acetic acid treatments on germination and vigour of carrot seeds.

Sample	Treatment	Germination Parameters (%)						Mean Germination Time (Days)	
		Germination Capacity		Abnormal Diseased Seedlings		Dead Seeds			
I	C ¹	44.0	a ²	35.7	d	11.7	ab	3.41	a
	W	52.0	ab	22.7	c	16.0	bc	3.36	a
	F	79.0	c	4.3	a	9.0	a	3.65	ab
	0.5%AA	57.0	b	19.3	bc	20.0	c	3.90	bc
	1%AA	49.3	ab	20.3	bc	21.0	c	4.18	c
	2%AA	49.3	ab	14.0	b	29.7	d	3.59	ab
II	C ¹	77.0	a	11.7	b	8.0	ab	3.10	a
	W	83.0	bc	5.3	a	9.0	bc	3.09	a
	F	85.0	c	2.7	a	5.3	a	3.55	bc
	0.5%AA	81.7	a–c	4.3	a	9.7	bc	3.31	ab
	1%AA	80.3	a–c	2.7	a	12.7	c	3.38	ab
	2%AA	79.3	ab	2.0	a	10.7	bc	4.87	c

¹ C—control (untreated seeds); W—seeds soaked in distilled water for 30 min; F—seeds treated with fungicide Zaprawa Nasienna T 75 WS/DS at a dose of 5 g kg^{−1} of seeds; 0.5%AA, 1%AA, 2%AA—seeds soaked in 0.5, 1 and 2% acetic acid solutions, respectively. ² Means in columns followed by the same letter, for each sample separately, are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

A reduction in the number of abnormal diseased seedlings was shown not only after fungicide and acetic acid treatments, but also after soaking seeds in distilled water. Soaking seeds in acetic acid solutions in some cases resulted in the deterioration of seed vigour (Table 1). An increase in mean germination time was observed when seeds of sample I were treated with 0.5 and 1% acetic acid, and after soaking seeds of sample II in 2% acetic acid solution.

The seeds of both samples were abundantly infested with fungi, especially *A. alternata*. This fungus was observed on 98.5 and 99.5% of untreated seeds in sample I and II, respectively (Table 2). Pathogenic *Alternaria* species, i.e., *A. dauci* and *A. radicina*, were also detected in both samples, however *A. radicina* prevailed. *Alternaria dauci* infected 12.0 and 2.0%, and *A. radicina* 59.5 and 17.5% of untreated seeds in sample I and II, respectively. Soaking seeds in the acetic acid solutions, regardless of concentration, resulted in an increase in the percentage of seeds free of fungi and a significant reduction in seed infestation with *A. alternata* and *A. radicina*, in both samples. In the case of sample I, treating seeds with 2% acetic acid controlled these fungi more effectively than fungicide, and additionally decreased seed infestation with *A. dauci*. None of the applied treatments affected the incidence of this fungus in sample II.

Table 2. Effects of acetic acid treatments on health of carrot seeds.

Sample	Treatment	Seed Infestation with Fungi (%)						Seeds Free of Fungi	
		<i>Alternaria alternata</i>		<i>Alternaria dauci</i>		<i>Alternaria radicina</i>			
I	C ¹	98.5	e ²	12.0	b	59.5	c	0	a
	W	99.0	e	9.0	b	49.0	c	0.5	a
	F	44.0	b	0	a	28.5	b	23.0	d
	0.5%AA	62.0	d	7.0	b	21.0	ab	3.5	b
	1%AA	68.5	c	7.5	b	22.5	ab	5.0	b
	2%AA	28.5	a	2.0	a	15.0	a	12.5	c
II	C ¹	99.5	d	2.0	a	17.5	b	0	a
	W	99.5	d	2.0	a	10.0	b	0	a
	F	53.5	a	1.0	a	1.0	a	38.0	d
	0.5%AA	89.0	c	0	a	2.0	a	7.0	b
	1%AA	71.0	b	1.5	a	0.5	a	24.0	c
	2%AA	60.0	ab	1.5	a	1.5	a	31.5	cd

^{1,2} For explanations see Table 1.

In sample I after 5 months of storage, irrespective of storage temperature, a deterioration of seed germination, compared to untreated seeds, was observed only if seeds were treated with 2% acetic acid (Table 3).

Table 3. Effects of acetic acid treatments on germination of stored carrot seeds—sample I.

Germination Parameters (%)	Storage Duration (Months)	Storage Temp. (°C)	Treatment												Mean	
			C ¹		W		F		0.5%AA		1%AA		2%AA			
Germination capacity	5	4	55.3	c ²	62.7	c	75.3	d	64.0	c	55.3	c	39.3	b	58.7	A
		20	46.0	b	60.7	c	72.7	d	73.7	d	62.0	c	25.3	a	56.7	A
		Mean	50.7	D	61.7	C	74.0	E	68.9	D	58.7	C	32.3	A		
	12	4	41.3	cd	65.7	f	69.0	g	67.7	fg	53.0	e	38.0	bc	55.8	B
		20	30.3	ab	48.0	de	75.3	g	69.7	fg	56.3	e	29.3	a	51.5	A
		Mean	35.8	A	56.9	B	72.2	C	68.7	C	54.7	B	33.7	A		
Abnormal diseased seedlings	5	4	23.7	ef	16.3	c–e	9.0	bc	19.0	de	14.0	cd	15.7	c–e	16.3	B
		20	31.7	f	21.7	d–f	3.0	a	6.0	ab	10.0	bc	10.7	bc	13.9	A
		Mean	27.7	D	19.0	C	6.0	A	12.5	B	12.0	B	13.2	B		
	12	4	29.3	f	16.7	cd	5.3	ab	9.7	bc	15.7	cd	18.7	de	15.9	B
		20	42.3	g	28.3	ef	3.3	a	5.7	ab	5.0	ab	5.7	ab	15.1	A
		Mean	35.8	D	22.5	C	4.3	A	7.7	AB	10.4	B	12.2	B		
Dead seeds	5	4	14.3	c–e	12.0	b–d	4.0	a	10.3	b–d	20.7	ef	28.0	f	14.9	A
		20	14.0	c–e	9.3	b–d	8.0	a–c	7.7	ab	15.3	de	45.0	g	16.6	A
		Mean	14.2	CD	10.7	BC	6.0	A	9.0	AB	18.0	D	36.5	E		
	12	4	22.7	f	8.7	b–d	5.7	ab	8.7	b–d	12.7	c–e	20.7	ef	13.2	A
		20	14.7	d–f	11.7	b–d	2.7	a	6.3	a–c	12.7	b–e	24.3	f	10.1	A
		Mean	18.7	D	10.2	BC	4.2	A	7.5	B	12.7	C	22.5	D		

¹ For explanation of abbreviations see Table 1. ² Means in the table followed by the same letter, for each storage period separately, are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

On the contrary, seeds treated with 0.5% acetic acid and stored at 20 °C were characterized by higher germination capacity than untreated seeds and seeds soaked in distilled water. Moreover, seeds treated with 1% acetic acid germinated better than untreated seeds. After 12 months of storage, regardless of the storage temperature, none of the applied treatments negatively affected germination capacity of sample I seeds. Furthermore, the treatment with 0.5% acetic acid improved seed germination capacity as effectively as the fungicide. The enhancement of this parameter was also observed if seeds were soaked in 1.0% acetic acid solution and in distilled water. After storage at 20 °C, irrespective of its duration, all applied acetic acid treatments, as well as the fungicide, significantly reduced the number of abnormal diseased seedlings compared with untreated seeds and seeds

soaked in distilled water. This reduction was also observed if seeds were stored at a lower temperature, especially for 12 months, but mostly in relation to untreated seeds. After 5 months of storage at both temperatures, a significant increase in the percentage of dead seeds was observed when seeds were treated with 2% acetic acid solution. Only in the case of seeds treated with 0.5% acetic acid and stored at 20 °C for 5 months, and at 4 and 20 °C for 12 months, were a lower number of dead seeds shown than in the control. In sample I, there were no significant differences between mean values of germination capacity for seeds stored at 4 and 20 °C for 5 months. After 12 months of storage, the mean value of this parameter for seeds preserved at 4 °C was higher than for seeds stored at 20 °C. Regardless of the storage duration, higher mean percentages of abnormal diseased seedlings were found if seeds were stored at a lower temperature. On the other hand, the temperature did not influence the percentage of dead seeds.

Seeds of sample II treated with 0.5 and 1% acetic acid, the fungicide and soaked in distilled water were characterized by significantly higher germination capacity than untreated seeds after 5 months of storage at 20 °C and 12 months of storage at 4 and 20 °C (Table 4). This phenomenon was also observed after 12 months of storage at 20 °C in the case of seeds treated with 2% acetic acid. Regardless of the storage temperature and duration, all applied acetic acid treatments, except seeds soaked in 0.5% acetic acid solution and stored for 5 months at 4 °C, resulted in a significant decrease in the percentage of abnormal diseased seedlings compared to untreated seeds. Moreover, after 12 months of storage, a lower percentage of dead seeds was recorded in acetic acid treatments numerous times compared with the control. This was shown for seeds treated with 0.5, 1 and 2% acetic acid solution and stored at 4 °C and for seeds treated with 1 and 2% acetic acid and stored at 20 °C. In sample II, regardless of the storage duration, there were no significant differences between mean values of germination capacity for seeds stored at 4 and 20 °C. After 5 months of storage, the temperature had no effect on the mean percentage of abnormal diseased seedlings, but after 12 months a higher mean percentage of these seedlings was observed if seeds were stored at 4 °C. On the other hand, a significantly higher mean percentage of dead seeds was demonstrated after 5 months of storage at 4 °C, but the temperature did not affect the percentages of these seeds after 12 months of storage.

Table 4. Effects of acetic acid treatments on germination of stored carrot seeds—sample II.

Germination Parameters (%)	Storage Duration (Months)	Storage Temp. (°C)	Treatment												Mean	
			C ¹		W		F		0.5%AA		1%AA		2%AA			
Germination capacity	5	4	76.3	a ^{a-c} ₂	82.3	cd	86.0	d	82.0	cd	83.0	cd	64.3	ab	79.0	A
		20	69.3	a	78.0	bc	91.3	e	83.7	cd	82.0	cd	73.0	ab	79.6	A
		Mean	72.8	A	80.2	B	88.7	C	82.9	B	82.5	B	68.7	A		
	12	4	64.7	ab	76.7	cd	83.7	de	83.7	c-e	83.0	c-e	73.7	bc	77.6	A
		20	60.0	a	81.7	c-e	86.7	e	84.0	e	86.7	e	75.7	cd	79.1	A
		Mean	62.4	A	79.2	BC	85.2	D	83.9	CD	84.9	CD	74.7	B		
Abnormal diseased seedlings	5	4	11.7	d	12.0	d	3.3	ab	7.7	cd	7.3	bc	5.0	bc	7.8	A
		20	21.3	e	10.0	cd	1.3	a	4.7	bc	4.7	bc	5.0	bc	7.8	A
		Mean	16.5	D	11.0	C	2.3	A	6.2	B	6.0	B	5.0	B		
	12	4	22.7	de	15.7	d	8.7	c	7.0	bc	6.3	bc	4.7	bc	10.9	B
		20	30.0	e	8.3	c	3.0	ab	5.3	bc	3.0	ab	1.3	a	8.5	A
		Mean	26.4	D	12.0	C	5.9	AB	6.2	B	4.7	AB	3.0	A		
Dead seeds	5	4	9.3	d	4.0	a-c	2.7	a-c	6.0	b-d	4.0	a-d	7.0	cd	5.5	B
		20	4.0	a-c	5.7	b-d	2.3	ab	3.0	a-c	2.0	a	5.7	b-d	3.8	A
		Mean	6.7	B	4.9	AB	2.5	A	4.5	AB	3.0	A	6.4	B		
	12	4	7.3	c	3.3	a-c	0.3	a	1.0	a	2.0	ab	2.0	ab	2.7	A
		20	5.3	bc	2.7	a-c	2.0	ab	1.3	ab	0.3	a	1.0	a	2.1	A
		Mean	6.3	B	3.0	A	1.2	A	1.2	A	1.2	A	1.5	A		

¹ For explanation of abbreviations see Table 1. ² Means in the table followed by the same letter, for each storage period separately, are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Seeds of sample I treated with 0.5% acetic acid solution and stored for 5 months at 4 °C were characterized by a lower value of mean germination time (MGT) than the control seeds (Table 5). Acetic acid at this concentration, irrespective of the storage duration and

temperature, did not negatively affect the speed of germination in relation to untreated seeds. In the case of sample II, after 5 months of storage at 4 °C, the lowest value of MGT was shown in seeds soaked in distilled water. Treating seeds with 0.5 and 1% acetic acid, regardless of the storage temperature, did not delay germination compared to untreated seeds. Deterioration of MGT was observed only if seeds were treated with 2% acetic acid solution. After 12 months of storage, acetic acid treatments generally negatively affected MGT compared to the control. Only in the case of seeds treated with 0.5% acetic acid and stored at a lower temperature, was an adverse effect on the speed of germination not observed. Seeds of both samples stored for 5 and 12 months at 4 °C germinated faster, based on the average values of mean germination time, than those which were preserved at 20 °C.

Table 5. Effects of acetic acid treatments on mean germination time of stored carrot seeds (days).

Sample	Storage Duration (Months)	Storage Temp. (°C)	Treatment												Mean	
			C ¹		W		F		0.5%AA		1%AA		2%AA			
I	5	4	3.18	b ²	3.38	bc	3.49	bc	3.11	a	4.24	d	4.64	e	3.67	A
		20	3.39	bc	3.33	bc	3.68	c	3.64	c	4.35	de	5.28	f	3.95	B
		Mean	3.29	A	3.36	A	3.59	B	3.38	A	4.30	C	4.96	D		
	12	4	3.25	ab	3.00	a	3.47	b	3.12	ab	4.15	c	4.50	d	3.58	A
		20	3.28	ab	3.14	ab	3.49	ab	3.52	b	4.32	cd	5.26	e	3.84	B
		Mean	3.27	AB	3.07	A	3.48	B	3.32	AB	4.24	C	4.88	D		
II	5	4	3.29	b	3.11	a	3.52	cd	3.43	bc	3.34	b	3.89	f	3.43	A
		20	3.55	cd	3.45	bc	3.85	ef	3.44	bc	3.73	de	4.45	g	3.75	B
		Mean	3.42	B	3.28	A	3.69	C	3.44	B	3.54	B	4.17	D		
	12	4	3.11	a	3.20	ab	3.45	cd	3.07	a	3.36	bc	4.00	e	3.37	A
		20	3.19	a	3.41	b–d	3.59	d	3.38	bc	3.49	cd	4.36	f	3.57	B
		Mean	3.15	A	3.31	BC	3.52	D	3.23	AB	3.43	CD	4.18	E		

¹ For explanation of abbreviations see Table 1. ² Means in the table followed by the same letter, for each storage period separately, are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Acetic acid and fungicide treatments, regardless of the temperature and duration of storage, significantly limited seed infestation with fungi of the genus *Alternaria* and increased the number of seeds free of fungi compared with untreated seeds and seeds soaked in distilled water (Table 6). Moreover, acetic acid, especially at concentrations of 1 and 2%, was frequently more effective, or as effective as the fungicide. It was especially observed after 12 months of storage in the case of *A. alternata* and *A. radicina*. Acetic acid treatments, irrespective of the storage duration, resulted in a significant increase in the percentage of seeds free of fungi. Furthermore, after 12 months of storage at 20 °C, seeds treated with 0.5, 1 and 2% acetic acid solutions were less infested with fungi than seeds treated with the fungicide. It was also observed in the case of seeds treated with 2.0% acetic acid after 12 months of storage at 4 °C. Temperature, regardless of the storage duration, significantly influenced mean percentages of seeds infested with fungi in sample I. The significantly lower mean percentages of seeds infested with *Alternaria* spp. and a higher mean percentage of seeds free of fungi was found after storage at 20 °C, than at 4 °C.

In sample II acetic acid treatments, irrespective of the storage period and temperature, resulted in a decrease in seed infestation with *A. alternata* in comparison with untreated seeds and seeds soaked in distilled water (Table 7). Moreover, treating seeds with 2% acid controlled this fungus, after storage at 4 °C, more efficiently than the fungicide. All applied acetic acid treatments reduced the number of seeds infested with *A. radicina*, after 5 and 12 months of storage, as effectively as the fungicide. Sample II seeds were characterized by a low infestation with *A. dauci* and none of the applied treatments affected the incidence of this fungus after 5 months of storage at 4 and 20 °C, and after 12 months of storage at 20 °C. However, after 12 months of storage at 4 °C all applied acetic acid treatments, as well as the fungicide and soaking seeds in distilled water, decreased significantly the number of seeds infected with this pathogen. Soaking in acetic acid solutions, regardless of the storage

duration and temperature, resulted in a significant increase in the percentage of seeds free of fungi, compared with untreated seeds and seeds soaked in distilled water. After 5 and 12 months of storage at 4 °C, in a 2% acetic acid treatment a higher percentage of seeds free of fungi was demonstrated than in the case of seeds treated with the fungicide.

Table 6. Effects of acetic acid treatments on health of stored carrot seeds—sample I.

Seed Infestation with Fungi (%)	Storage Duration (Months)	Storage Temp. (°C)	Treatment												Mean	
			C ¹		W		F		0.5%AA		1%AA		2%AA			
<i>Alternaria alternata</i>	5	4	98.5	h ²	95.5	gh	18.0	cd	66.0	f	29.5	de	35.5	e	57.2	B
		20	92.5	g	76.0	f	7.0	ab	12.0	bc	3.0	a	2.5	a	32.2	A
		Mean	95.5	D	85.8	C	12.5	A	39.0	B	16.3	A	19.0	A		
	12	4	100.0	h	93.5	g	24.0	d	21.5	d	11.0	c	12.0	c	43.7	B
		20	81.5	f	47.0	e	4.5	b	1.5	ab	2.0	ab	0	a	22.8	A
		Mean	90.8	E	70.3	D	14.3	C	11.5	BC	6.5	AB	6.0	A		
<i>Alternaria dauci</i>	5	4	11.0	d	9.0	cd	1.0	ab	11.0	d	2.5	ab	4.0	bc	6.4	B
		20	7.5	cd	8.5	cd	3.0	bc	0.5	a	1.5	ab	2.5	ab	3.9	A
		Mean	9.3	B	8.8	B	2.0	A	5.8	A	2.0	A	3.3	A		
	12	4	8.5	d	8.0	d	2.5	bc	5.0	bc	6.5	ac	3.5	bc	5.7	B
		20	12.0	d	7.5	cd	1.0	ab	0.5	ab	0	a	0	a	3.5	A
		Mean	10.3	B	7.7	B	1.8	A	2.8	A	3.3	A	1.8	A		
<i>Alternaria radicina</i>	5	4	47.0	e	34.0	d	16.5	c	18.0	c	13.0	bc	18.0	c	24.4	B
		20	54.5	e	38.5	de	13.5	bc	11.0	a-c	8.5	ab	6.0	a	22.0	A
		Mean	50.8	C	36.3	B	15.0	A	14.5	A	10.8	A	12.0	A		
	12	4	62.5	d	35.5	c	12.5	b	14.5	b	10.0	b	9.0	b	24.5	B
		20	75.0	e	29.0	c	12.0	b	3.0	a	2.5	a	3.0	a	20.8	A
		Mean	68.8	D	32.3	C	12.3	B	8.8	A	6.3	A	6.0	A		
Seeds free of fungi	5	4	0	a	0.5	a	58.5	d	12.0	b	13.0	b	35.5	c	20.4	A
		20	0	a	9.0	b	67.0	e	65.0	e	66.0	e	73.5	e	46.8	B
		Mean	0	A	4.8	B	62.8	D	38.5	C	39.5	C	54.5	D		
	12	4	0	a	0.5	a	53.5	c	50.0	c	60.5	c	73.0	d	39.6	A
		20	0	a	36.0	b	75.0	d	91.0	e	89.0	e	93.0	e	64.0	B
		Mean	0	A	18.3	B	64.3	C	70.5	D	74.8	D	83.0	E		

¹ For explanation of abbreviations see Table 1. ² Means in the table followed by the same letter, for each storage period separately, are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Table 7. Effects of acetic acid treatments on health of stored carrot seeds—sample II.

Seed Infestation with Fungi (%)	Storage Duration (Months)	Storage Temp. (°C)	Treatment												Mean	
			C ¹		W		F		0.5%AA		1%AA		2%AA			
Alternaria alternata	5	4	100.0	g ²	96.5	f	32.0	cd	40.0	d	31.5	cd	17.0	ab	52.8	B
		20	100.0	g	62.5	e	10.0	a	29.0	cd	22.5	bc	14.5	ab	39.8	A
		Mean	100.0	D	79.5	C	21.0	A	34.5	B	27.0	B	15.8	A		
	12	4	98.5	d	81.5	c	29.5	b	30.5	b	25.0	b	5.0	a	45.0	B
		20	93.5	c	29.0	b	1.5	a	4.5	a	3.0	a	5.0	a	22.8	A
		Mean	96.0	D	55.3	C	15.5	B	17.5	B	14.0	B	5.0	A		
Alternaria dauci	5	4	2.0	a	0.5	a	1.5	a	1.5	a	0	a	0	a	0.9	A
		20	2.5	a	3.0	a	0.5	a	0.5	a	1.0	a	0	a	1.3	A
		Mean	2.3	A	1.8	A	1.0	A	1.0	A	0.5	A	0	A		
	12	4	4.5	b	1.5	a	0.5	a	1.5	a	0	a	1.5	a	1.6	B
		20	0.5	a	0.5	a	0	a	0.5	a	0	a	0	a	0.3	A
		Mean	2.5	B	1.0	A	0.3	A	1.0	A	0	A	0.8	A		
Alternaria radicina	5	4	16.5	ef	9.0	c–e	1.0	a	3.5	a–d	3.0	a–c	1.5	ab	5.8	A
		20	25.0	f	9.0	de	5.5	b–d	1.5	ab	1.5	ab	4.0	a–d	7.8	A
		Mean	20.8	C	9.0	B	3.3	A	2.5	A	2.3	A	2.8	A		
	12	4	23.0	d	10.5	c	3.0	ab	2.0	ab	1.0	a	1.5	a	6.8	B
		20	19.5	d	5.5	bc	0	a	1.0	a	3.0	ab	0	a	4.8	A
		Mean	21.3	C	8.0	B	1.5	A	1.5	A	2.0	A	0.8	A		

Table 7. Cont.

Seed Infestation with Fungi (%)	Storage Duration (Months)	Storage Temp. (°C)	Treatment												Mean	
			C ¹		W		F		0.5%AA		1%AA		2%AA			
Seeds free of fungi	5	4	0	a	1.0	a	46.5	bc	44.5	bc	52.5	cd	65.5	de	35.0	A
		20	0	a	32.5	b	79.5	f	56.5	c-e	58.0	c-e	69.0	ef	49.3	B
		Mean	0	A	16.8	B	63.0	DE	50.5	C	55.3	CD	67.3	E		
	12	4	0	a	7.5	b	59.0	c	60.5	c	67.0	c	83.0	d	46.2	A
		20	1.5	a	66.5	c	97.0	e	86.0	d	86.5	d	90.0	d	71.3	B
		Mean	0.8	A	37.0	B	78.0	DE	73.3	C	76.8	CD	86.5	E		

¹ For explanation of abbreviations see Table 1. ² Means in the table followed by the same letter, for each storage period separately, are not significantly different at a level $\alpha = 0.05$, according to Duncan's multiple range test.

Seeds of sample II, stored for 5 and 12 months at 20 °C were infested with *A. alternata* to a lower extent, based on mean percentages of infested seeds, than seeds stored at 4 °C. In the case of *A. dauci* and *A. radicina* this phenomenon was observed only after 12 months of storage. Moreover, after storage at a higher temperature a significantly lower mean percentage of seeds free of fungi was detected, regardless of the storage duration.

4. Discussion

Acetic acid, because of antifungal and antibacterial properties, has been commonly used in food production as a preservative and disinfectant [21–24]. These properties encouraged scientists, who have also been trying to use it for plant protection, including seed treatment [5–13,25]. Various methods of acetic acid application have been proposed to control seed-borne pathogens, e.g., fumigation in acetic acid vapour, mixing seeds with a specific amount of the acid, or soaking seeds in the acid solutions. Our previous experiments, as well as present results, revealed that soaking in acetic acid solutions effectively controlled fungi of the genus *Alternaria* associated with carrot seeds [13]. Moreover, the present experiment showed that the positive effect of acetic acid treatment persisted for short- (5 months) and long-term (12 months) storage. Generally, before and after storage, regardless of the temperature, seeds of both samples treated with acetic acid were characterized by a lower infestation with *A. alternata* and *A. radicina* than untreated seeds and seeds soaked in distilled water. Additionally, acetic acid often controlled these fungi more effectively than the fungicide. The treatment was also effective against *A. dauci*, however the decrease in seed infestation was most significant after storage, in the case of sample I, which was characterized by a higher level of initial seed infection with this fungus than sample II. Generally, especially after storage, an increase in the concentration of acetic acid resulted in a significant increase in the number of seeds free of fungi, and a decrease in seed germination. Carrot seeds soaked in 0.5 and 1% acetic acid solutions showed higher germination capacity after storage than untreated seeds, while, treating seeds with 2% acetic acid solution often adversely affected their germination and vigour. Previous experiments confirmed that too high a concentration of acetic acid may be phytotoxic to plants and seeds. Pasini et al. [26], who studied effectiveness of acetic acid against rose powdery mildew (*Sphaerotheca pannosa* var. *rosae* Woron., syn. *Podosphaera pannosa* (Wallr.) de Bary) in a glasshouse, found that white vinegar, applied at 5 and 10% concentrations gave good disease control, but 0.25 and 0.5% acetic acid was phytotoxic. Sholberg et al. [7] observed that fumigation of wheat seeds with acetic acid vapour at 2 and 4 g kg^{−1} of seeds controlled common bunt (*T. tritici* and *T. laevis*) in the field as effectively as the fungicide, but acetic acid applied at the higher dose, i.e., 4 g kg^{−1}, caused the reduction of tiller numbers. Szopińska [10] reported that soaking in 1, 2.5 and 5% acetic acid solution significantly decreased zinnia seed infestation with *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp. and *Gonatobotrys simplex* Corda (syn. *Melanospora simplex* (Corda) D. Hawksw.), however, regardless of the concentration, the treatments decreased the total number of germinating seeds and germination capacity, increased the number of abnormally deformed seedlings, and prolonged germination. Despite of these observations, there are some reports that a properly applied acetic acid treatment may improve seed germination and storability.

El-Saidy and El-Hai [11] found that dipping of sunflower seeds in acetic acid resulted in an increase in seed germination percentage and vigour, before and after 6 months storage. Moreover, the treatment reduced the incidence of several fungal genera, i.e., *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Stemphylium*, *Trichothecium* and *Verticillium*, before and after storage. In the aspect of seed storage, protection of the seeds from storage fungi, i.e., *Aspergillus* spp. and *Penicillium* spp. is an additional benefit of acetic acid treatment, because these fungi are considered as the main reason for seed deterioration. This possibility was also examined by Sholberg and Gaunce [9], who treated canola, corn, rice and wheat seeds, artificially inoculated with *A. flavus*, with acetic acid vapour. The authors reported that fumigation of wheat seeds with acetic acid at 0.78 mL kg⁻¹ of seeds resulted in the improvement of seed germination and the complete eradication of *A. flavus* after 102 days of storage at 20 °C. This fungus was also not detected on canola seeds treated with acetic acid at 0.58 mL kg⁻¹ of seeds after 38 days of storage at the same temperature.

In the present experiment seed-borne fungi were significantly affected by the storage temperature. Seeds stored at 4 °C were characterized by a higher seed infestation with fungi than those stored at 20 °C. Low temperature, optimal for seed storage, usually also favours pathogens viability [27]. Therefore, the reduction of temperature may increase the length of survival of seed-borne microorganisms, however, some pathogens, especially in the case of short-term storage, may not respond to this factor [28]. We observed this phenomenon in sample II after 5 months of storage in relation to seed infestation with *A. dauci* and *A. radicina*. Nevertheless, after 12 months, seeds stored at a lower temperature were characterized by a higher level of infection with these fungi. *Alternaria* spp. are regarded as long-living fungi, and conditions favouring seed viability are also conducive to pathogens survival [2,29]. Therefore, the possibility of the control of these fungi during storage with a cheap and safe for the environment organic compound, seems to be very promising.

5. Conclusions

Regardless of the initial seed quality and the storage temperature, soaking carrot seeds in 0.5 and 1% acetic acid solutions effectively reduced their infestation with *Alternaria* spp. and improved seed germination capacity compared to untreated seeds, therefore these treatments may be recommended for controlling fungi during seed storage. However, it should be considered that the lower (4 °C) storage temperature favours seed infestation with *Alternaria* spp., and beneficial effects of acetic acid treatment might be clearer at 20 °C, especially in the case of seeds characterized by the higher initial incidence of fungi.

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