Rosińska AA (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 2, Article number 12631
DOI:10.15835/nbha50212631
Research Article

Effect of acetic acid treatments and storage on the quality of lettuce (*Lactuca sativa* L.) seeds

Agnieszka A. ROSIŃSKA

Poznań University of Life Sciences, Department of Phytopathology, Seed Science and Technology, Dąbrowskiego 159, Poznań, Poland; agnieszka.rosinska@up.poznan.pl

Abstract

Seedborne fungi negatively affect the quality of lettuce (*Lactuca sativa* L.) seeds and they are impact on their storability. Using the acetic acid can be an alternative to chemical treatment. Seeds were soaked for 30 minutes in a solution of acetic acid at concentrations of 0.25%, 0.5%, and 2%. Untreated seeds, seeds soaked for 30 minutes with distilled water and seeds treated with Dithane NeoTec 75 were controls. Seeds germination, vigour and seed health before and after 18 months of storage at 4 and 20 °C were tested. Before storage the acetic acid solution at concentrations of 0.25 and 0.5% significantly decreased number of dead seeds and increased germination at first and final counts, 2.0% solution worsened germination and vigour of seeds of both samples. Generally acetic acid solutions limited occurrence of fungi. Seeds stored at a lower temperature (4 °C) were characterized by significantly higher percentages of total germinating seeds (Gmax) and percentage of germination at first and final counts. Deterioration of the germination at first and final counts was found only after soaking the seeds in acetic acid solution at a concentration of 2%. Acetic acid solutions at concentration 0.5 and 2.0% effectively reduced occurrence of *Alternaria alternata*.

Keywords: acetic acid; germination capacity; seed health; seed quality; seed vigour; seed treatment; storage

Introduction

Seedborne fungi are responsible for pre- and postemergence seed death, affect seed vigour and germination (Tsedaley, 2015), they are also impact on the storability of seeds. Fungi associated with lettuce seeds (achenes), as *Alternaria* spp., *Botrytis cinerea* and *Fusarium* spp., mainly contamined seed surface or occupied the integuments, pericarp and endosperm. After the seed priming pathogenic and saprophytic fungi can penetrated inner tissues as integuments or embryo (Szopińska and Tylkowska, 2003). In addition, storage fungi, which include *Aspergillus* spp. and *Penicillium* spp., develop when seeds are stored at inappropriate temperatures and humidity. Fungi can infect seeds before harvesting or immediately after, and they also produce mycotoxins. Fungal secondary metabolites can negatively affect seed germination, viability, vigour and seedlings emergence (Ismaiel and Papenbrock, 2015). Khairnar *et al.* (2011) reported that germination of cereals seeds was suppressed by presence of mycotoxins.

Received: 18 Jan 2022. Received in revised form: 03 Mar 2022. Accepted: 07 Mar 2022. Published online: 23 May 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

In the chemical protection of lettuce seeds against seedling damping-off, a broad-spectrum contact fungicide is used. That kind of fungicides are effective only to spores, which are on seed surface, they are not work on internal fungal infections (Mancini and Romanazzi, 2014). Moreover, chemical treatment of seeds, negatively affects the environment. The acetic acid is commonly used as preservative and has antimicrobial properties. Szopińska (2013) and van der Wolf *et al.* (2008) indicate that the strong antifungal action of acetic acid may be useful in seed treatment and disinfection as an alternative or in combination with physical treatments. According to Sholberg and Gaunce (1995), acetic acid vapour at low concentrations is an excellent biocide, kills fungal spores, does not damage the surface of fumigated fruit, and is effective at low temperatures, making it suitable for use in cold storage. Acetic acid vapour interferes a transport in microbial cells, penetrates microbial cells and has a toxic effect on them (Banwart, 1989).

Because of the short growing season and lettuce's ability to accumulate toxic substances, lettuce protection should focus on the use of non-chemical methods that are harmless to human health and the environment. Promising results obtained by other authors investigating the effect of acetic acid on seed quality of different plant species contributed to the research in the present study. The aim of this study was therefore to determine the effect of treating lettuce seeds with acetic acid on their quality, before and after storage.

Materials and Methods

Material

Two standard lettuce seed samples cv. Królowa Majowych (sample I – lot No PL 704/63/51/388/A, sample II – lot No PL 704/63/51/389/A), obtained from TORSEED Seed Company in Toruń, were used in the experiment. Acetic acid (99.8%) produced by Sigma-Aldrich Co. was applied for seed treatment. Additionally, fungicide Dithane Neo Tec 75 WG (a.i. 75% mancozeb), produced by TARGET Sp. J. in Wałbrzych, was used as an alternative chemical control.

Seed treatment and storage

Seeds were treated by soaking in 0.25, 0.5 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 Dithane Neo Tec 75 WG at a dose 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 high-constant-temperature oven method (ISTA, 2020). Depending on the treatment, it ranged from 6.9 to 7.2% in sample I and from 6.6 to 7.3 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 18 months of storage.

Seed germination and vigour tests

Germination and vigour tests were performed separately for six replications of 50 seeds from each treatment. The seeds were incubated in 9 cm diameter Petri dishes on six layers of filter paper, moistened with distilled water, 50 seeds per dish, in darkness at 20 °C. Percentages of normal seedlings (germination at first count) was calculated after four days of incubation. Other parameters: percentage of normal seedlings (germination of final count), abnormal diseased seedlings, abnormal deformed seedlings, fresh seeds and dead seeds were calculated after seven days of incubation according to ISTA rules (ISTA, 2020). Additionally, the percentage of germinating seeds (Gmax) was calculated on the base of seed vigour test.

The vigour of the seeds was assessed by determining the germination speed (T75 - time to 75% of Gmax and MGT - mean germination time) and germination uniformity (U90-10 - time between 10 and 90% of Gmax). Germinating seeds were counted daily and removed from the plate, until no new germs appeared. Seeds with the radicle at least 1 mm long were considered as germinating.

Seed health test

Deep-freeze blotter test was performed on five replications of 40 seeds from each treatment. Seeds were incubated in 9 cm diameter Petri dishes on six layers of filter paper, 20 seeds per dish, for one day at 20 °C in darkness, then transferred to -20 °C for 24 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 (Watanabe, 2002; Mathur and Kongsdal, 2003). Additionally, the percentage of seeds free of fungi was evaluated.

Statistical analysis

SeedCalculator version 2.1 software (Jalink and van der Schoor, 1999) was applied to analyze 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 18 months of storage. Duncan's multiple range test was applied to estimate the differences between the means at a level $\alpha = 0.05$.

Results

Before storage

Tested samples varied in seed germination. The percentage of germinating seeds (Gmax) of untreated seeds before storage was 97.7 and 85.3%, respectively for sample I and sample II. Germination at the first and the final counts of untreated seeds (control) of sample I were higher than sample II. Appling acetic acid at concentration of 2.0% decreased these parameters compared with control. Untreated seeds of sample II were also characterized by a high percentage of dead seeds (21.7%). However, using acetic acid solution at concentrations of 0.25 and 0.5% significantly decreased number of dead seeds and increased germination at first and final counts (Table 1).

Table 1. Germination of untreated and treated seeds of samples I and II before storage (%)

	Seed treatments					
	C[1]	W	F	0.25A	0.5A	2.0A
	Sample I					
Percentage of total germinating seeds Gmax	97.7 b[2]	97.3 b	99.3 b	98.7 b	99.0 b	10.0 a
Germination at the first count	94.0 c	93.7 c	92.7 c	92.0 c	87.0 b	7.0 a
Germination at the final count	94.0 c	94.3 c	92.7 c	92.0 c	87.0 c	10.7 a
Abnormal diseased seedlings	2.0 a	2.0 a	4.3 ab	4.7 ab	8.7 b	22.7 c
Abnormal deformed seedlings	1.0 a	0.7 a	0.3 a	0 a	1.0 a	10.3 b
Fresh seeds	0 a	0 a	0 a	0 a	0 a	30.3 b
Dead seeds	3.0 a	3.0 a	2.7 a	3.3 a	3.3 a	26.0 b
	Sample II					
Percentage of total germinating seeds Gmax	85.3 b	84.0 b	84.7 b	84.7 b	83.7 b	16.3 a
Germination at the first count	61.0 b	75.3 c	72.7 c	79.3 c	74.7 c	13.0 a
Germination at the final count	64.7 a	76.7 c	72.7 bc	79.7 c	74.7 c	17.3 a

Abnormal diseased seedlings	10.7 c	4.3 b	7.0 bc	4.0 b	0.7 a	4.7 b
Abnormal deformed seedlings	1.7 a	2.0 ab	1.7 ab	0.3 a	6.0 b	12.3 c
Fresh seeds	1.3 ab	0 a	1.3 ab	0.7 ab	2.3 b	35.0 c
Dead seeds	21.7 a-c	17.0 ab	17.3 ab	15.3 a	16.3 a	30.7 c

[1]C – untreated seeds (control); W – seeds soaked I distilled water for 30 min; F – seeds treated with fungicide Dithane NeoTec 75 WP at a dose 5 g•kg^{-1} of seeds; 0.25A, 0.5A, 2.0 A – seeds soaked for 30 min in 0.25, 0.5 and 2% acetic acid solutions, respectively.
[2]Means in rows followed by the same letter, for each sample separately, are not significantly different at a level α = 0.05, according to Duncan's multiple range test.

Seeds of sample II germinated faster and more uniform than seeds of sample I. An elongation of seed germination time was observed along with an increase of acetic acid solution concentration. The treatment of lettuce seeds with a 0.25% acetic acid solution did not deteriorate either the germination speed or germination uniformity compared to all controls (Table 2).

Table 2. Vigour of untreated and treated seeds of samples I and II before storage (days)

	Seed treatments					
	C[2]	W	F	0.25A	0.5A	2.0A
	Sample I					
T_{75}[1]	1.05 a[3]	0.61 a	1.08 a	0.89 a	1.63 b	6.35 c
U_{90-10}	1.29	1.55 a	1.34 a	1.68 ab	2.26 b	2.34 b
MGT	0.81 a	0.5 a	0.83 a	0.6 a	1.26 b	5.71 c
	Sample II					
T_{75}	0.29 a	0.2 a	0.5 a	0.24 a	1.3 b	6.46 c
U_{90-10}	0.99 a	0.73 a	1.22 a	0.8 a	1.07 a	2.03 b
MGT	0.35 a	0.26 a	0.4 a	0.27 a	1.1 b	5.91 c

[1]T_{75} – time to 75 % of Gmax
U_{90-10} – time between 10 and 90 % of Gmax
MGT – mean germination time
[2]For explanations see Table 1
[3]Means in rows followed by the same letter, for each sample separately, are not significantly different at a level α = 0.05, according to Duncan's multiple range test.

The seeds of sample I were most often occupied by the following fungi: *Alternaria alternata* (Fr.) Keissler (83.0%), *Cladosporium* spp. (38.0%) and *Fusarium* spp. (66.0%), and sample II by *A. alternata* (86.0%), *Fusarium* spp. (41.0%), *Melanospora simplex* (Corda) D. Hawskw (77.0%) and *Stemphylium botryosum* Wallr. (37.5%). Treatment of seeds with the solution of acetic acid with the highest concentration worsened the germination and vigour of seeds of both samples. However, this solution has most effectively reduced the occurrence of fungi on seeds, with the exception of *Cladosporium* spp., on which it had a stimulating effect. The increase in seed settlement by *Cladosporium* spp. after application of 2.0% acetic acid solution for sample I was 9.5% and for sample II 26.0% compared to untreated seeds. Solutions at concentrations 0.5 and 2.0% were as effective or more effective than the fungicide in reducing the incidence of *A. alternata, B. cinerea, M. simplex, Fusarium* spp. and *S. botryosum* on seeds (Table 3).

Table 3. Seed health of untreated and treated seeds of samples I and II before storage (%)

	Seed treatments					
	C[1]	W	F	0.25A	0.5A	2.0A
	Sample I					
Alternaria alternata	83.0 f[2]	52.5 d	68.5 e	31.0 c	12.5 b	6.5 a
Botrytis cinerea	5.5 cd	0.5 ab	10.0 d	3.5 bc	0 a	0 a
Cladosporium spp.	38.0 b	20.0 a	34.0 b	14.5 a	43.5 bc	47.5 c
Fusarium spp.	66.0 d	81.5 e	45.0 c	49.5 c	28.5 b	4.0 a
Melanospora simplex	8.0 ab	1.0 a	6.0 b	1.0 a	0 a	0.5 a
Stemphylium botryosum	12.5 c	3.0 b	4.0 b	2.0 ab	1.5 ab	1.0 a
Seeds free of fungi	0 a	0 a	0 a	4.0 a	14.0 b	22.0 b
	Sample II					
Alternaria alternata	86.0 b	99.0 c	98.5 bc	94.5 bc	26.5 a	20.0 a
Botrytis cinerea	3.5 bc	5.0 cd	7.5 d	16.5 e	0.5 a	1.0 ab
Cladosporium spp.	17.0 bc	6.0 a	12.5 ab	8.0 ab	28.5 cd	43.0 d
Fusarium spp.	41.0 b	73.0 e	44.5 bc	61.0 de	57.5 cd	1.5 a
Melanospora simplex	77.0 c	60.0 b	52.5 b	1.5 a	0 a	0.5 a
Stemphylium botryosum	37.5 cd	49.5 d	34.0 c	5.5 b	3.0 ab	1.0 a
Seeds free of fungi	0 a	0 a	0 a	0 a	6.0 b	11.5 c

[1, 2] For explanations see Table 1

After storage

Seeds stored at a lower temperature (4 °C) were characterized by significantly higher percentages of total germinating seeds (Gmax) and percentage of germination at first and final counts. Deterioration of the germination at first and final counts was found only after soaking the seeds in acetic acid solution at a concentration of 2%, irrespective of the storage temperature of the seeds of sample I. The other treatments did not affect these parameters. For sample II treatment with 0.25 and 2% acetic acid solutions generally reduced seeds germination at the first and final counts. However, it was observed that soaking the seeds in 0.25% acetic acid solution and storage at a lower temperature did not deteriorate these parameters. Treatment of sample I seeds with 0.25% solution did not increase the percentage of abnormal deformed seedlings compared to untreated and fungicide treated seeds. The significant increase in fresh seeds was observed when sample I seeds where soaked in distilled water, treated with fungicide and treated with acetic acid solutions. However, the highest value of that parameter was observed when 2% acetic acid solution was used. Significantly more abnormal deformed seedlings and fresh seeds were observed after storing seeds of sample II at higher temperature. As in the case of sample I, the solution of the highest concentration of acetic acid caused a significant increase in the percentage of deformed abnormal seedlings and fresh seeds. More abnormal deformed seedlings and fresh seeds were also noted after soaking the seeds with 0.25% acetic acid solution and in distilled water, however, there were significantly less of them than after soaking in 2% acetic acid. No effect of treatment on the occurrence of seedlings with disease symptoms was found in both samples tested. However, a significantly higher percentage of these seedlings was observed after storage of sample II seeds at 4 °C. Significantly lower dead seeds were observed after storage at a higher temperature and after treatment of the seeds with 0.5% acetic acid solution, and in the case of sample I also after fungicide usage (Tables 4).

Table 4. Germination of untreated and treated lettuce seeds of samples I and II after 18 months of storage (%)

	Temperature [°C]	Seed treatment						Mean for temperature
		C[1]	W	F	0.25A	0.5A	2.0A	
		Sample I						
Percentage of total germinating seeds Gmax	4	82.2 b-d[2]	86.7 d	79.3 b	81.7 b-d	84.7 cd	79.0 b	82.3 B
	20	85.3 d	79.7 bc	85.0 cd	83.3 b-d	77.7 b	49.3 a	76.7 A
	Mean for treatment	83.8 B	83.2 B	82.2 B	82.5 B	81.2 B	64.2 A	
Germination at the first count	4	72.3 c	80.0 d	73.0 cd	72.0 c	70.6 c	33.7 b	66.9 B
	20	75.0 cd	70.0 c	68.0 c	70.0 c	71.0 c	14.7 a	61.5 A
	Mean for treatment	73.7 B	75.0 B	70.5 B	71.0 B	70.8 B	24.2 A	
Germination at the final count	4	75.3 c	84.0 d	79.3 cd	75.0 c	74.3 c	50.3 b	73.0 B
	20	82.0 cd	75.3 c	76.3 c	74.0 c	78.3 cd	25.3 a	68.5 A
	Mean for treatment	78.7 B	79.7 B	77.8 B	74.5 B	76.3 B	37.8 A	
Abnormal diseased seedlings	4	3.7 a	0.7 a	0.3 a	2.3 a	1.3 a	1.0 a	1.6 A
	20	1.0 a	0.3 a	0.7 a	1.0 a	1.0 a	1.3 a	0.9 A
	Mean for treatment	2.4 A	0.5 A	0.5 A	1.7 A	1.2 A	1.2 A	
Abnormal deformed seedlings	4	2.0 a	4.0 a	2.3 a	3.0 a	5.3 a	17.7 b	5.7 A
	20	2.3 a	4.3 a	2.3 a	4.3 a	4.0 a	16.0 b	5.5 A
	Mean for treatment	2.2 A	4.2 AB	2.3 A	3.7 AB	4.7 B	16.9 C	
Fresh seeds	4	2.0 a	1.0 a	12.0 c	1.3 a	6.3 b	13.3 c	6.0 A
	20	4.0 b	13.0 c	16.3 c	13.3 c	14.3 c	16.0 d	12.8 B
	Mean for treatment	3.0 A	7.0 B	14.2 C	7.3 B	10.3 C	14.7 D	
Dead seeds	4	17.0 ef	10.3 c-e	6.0 a-c	18.3 f	12.7 d-f	17.7 f	13.7 B
	20	10.7 d-f	7.0 a-d	4.3 ab	7.3 b-d	2.3 a	11.7 d-f	7.2 A
	Mean for treatment	13.9 C	8.7 B	5.2 A	12.8 C	7.5 AB	14.7 C	
		Sample II						
Percentage of total germinating seeds Gmax	4	98.0 e	96.3 c-e	97.0 c-e	97.3 de	95.3 b-d	92.7 bc	96.1 B
	20	97.0 c-e	93.7 bc	97.7 de	91.0 b	96.3 c-e	67.3 a	90.5 A
	Mean for treatment	97.5 C	95.0 BC	97.4 BC	94.2 B	95.8 BC	80.0 A	
Germination at the first count	4	90.0 ef	90.3 ef	89.7 ef	88.7 ef	89.3 ef	16.3 b	77.4 B
	20	87.7 de	64.0 c	93.7 f	69.3 c	81.7 d	9.0 a	67.6 A
	Mean for treatment	88.9 CD	77.2 B	91.7 D	79.0 B	85.5 C	12.7 A	
Germination at the final count	4	90.7 de	94.3 de	92.3 de	91.3 de	91.7 de	42.7 b	83.8 B
	20	91.0 de	78.7 c	96.0 e	74.7 c	88.7 d	23.7 ab	75.5 A
	Mean for treatment	90.9 CD	86.5 BC	94.2 D	83.0 B	90.2 CD	33.2 A	
Abnormal diseased seedlings	4	1.7 a	1.3 a	2.3 a	3.7 a	1.3 a	3.0 a	2.2 B
	20	2.0 a	0.3 a	0.3 a	1.3 a	1.3 a	0 a	0.9 A
	Mean for treatment	1.9 A	0.8 A	1.3 A	2.5 A	1.3 A	1.5 A	
Abnormal deformed seedlings	4	2.7 ab	3.3 ab	1.3 a	2.0 a	3.7 ab	37.7 e	8.5 A
	20	3.0 ab	13.0 c	1.0 a	12.0 c	5.7 b	26.7 d	10.2 B
	Mean for treatment	2.9 AB	8.2 C	1.2 A	7.0 C	4.7 BC	32.2 D	
Fresh seeds	4	0 a	0 a	0.3 a	0 a	0.3 a	14.7 d	2.6 A

	20	0.7 ab	5.3 c	1.7 b	11.7 d	4.0 c	48.0 e	11.9 B
	Mean for treatment	0.4 A	2.7 C	1.0 AB	5.9 D	2.2 BC	31.4 E	
Dead seeds	4	5.0 c	1.0 a-c	3.7 bc	3.0 bc	0.3 a	2.0 ab	2.5 B
	20	3.3 a-c	2.7 a-c	1.0 a-c	0.3 a	0.3 a	1.7 a-c	1.6 A
	Mean for treatment	4.2 B	1.9 AB	2.4 AB	1.7 AB	0.3 A	1.9 AB	

[1]For explanations see Table 1

[2]Means in the table followed by the same letter, for each sample separately, are not significantly different at a level α = 0.05, according to Duncan's multiple range test

The effect of treating seeds with acetic acid solutions was different for both samples. The seeds of sample I were characterized by worse vigour than the seeds of sample II. It was found that after soaking with 0.25 and 0.5% acetic acid solutions, they germinated faster (T75, MGT) than seeds untreated and treated with fungicide. There were no differences in germination uniformity after treating seeds with these solutions compared to control combinations. In case of sample II, no germination acceleration was found. Moreover, mean germination time of seeds soaked in 0.25% acetic acid solution has significantly extended compared to the control. Significant deterioration of germination speed and uniformity of seeds of both samples, irrespective of storage temperature, was observed after application of acetic acid of the highest concentration. Seeds of both samples were characterized by better vigour after storage at 4 °C (Table 5).

Table 5. Vigour of untreated and treated lettuce seeds of samples I and II after 18 months of storage (days)

	Temperature [°C]	Seed treatment						Mean for temperature
		C[1]	W	F	0.25A	0.5A	2.0A	
		Sample I						
T_{75}[2]	4	1.20 b[3]	0.43 a	1.01 b	0.27 a	0.36 a	2.80 c	1.01 A
	20	1.20 b	1.01 b	1.10 b	0.87 b	1.01 b	3.48 d	1.45 B
	Mean for treatment	1.20 B	0.72 A	1.06 B	0.57 A	0.69 A	3.14 C	
U_{90-10}	4	1.08 ab	1.38 ab	1.36 ab	0.92 a	1.17 ab	3.63 c	1.59 A
	20	1.67 ab	1.61 ab	1.80 b	1.82 b	1.78 b	4.49 c	2.20 B
	Mean for treatment	1.38 A	1.50 A	1.58 A	1.37 A	1.48 A	4.06 B	
MGT	4	0.98 d	0.46 ab	0.76 b-d	0.32 a	0.39 a	2.43 e	0.88 A
	20	0.90 cd	0.73 b-d	0.77 cd	0.62 bc	0.68 b-d	2.91 f	1.10 B
	Mean for treatment	0.94 C	0.60 AB	0.77 B	0.47 A	0.54 A	2.63 D	
Sample II								
T_{75}	4	0.33 a	0.14 a	0.17 a	0.12 a	0.17 a	3.83 c	0.79 A
	20	0.33 a	0.84 b	0.33 a	0.95 b	0.78 b	4.98 d	1.37 B
	Mean for treatment	0.33 AB	0.49 AB	0.25 A	0.54 B	0.48 AB	4.41 C	
U_{90-10}	4	0.83 a	0.54 a	0.58 a	0.46 a	0.64 a	3.53 c	1.10 A
	20	0.88 a	2.12 b	1.04 a	2.15 b	1.96 b	4.32 d	2.08 B
	Mean for treatment	0.86 A	1.33 B	0.81 A	1.31 B	1.30 B	3.93 C	
MGT	4	0.27 a	0.21 a	0.20 a	0.17 a	0.27 a	2.99 c	0.69 A
	20	0.28 a	0.69 b	0.34 a	0.71 b	0.63 b	3.91 d	1.09 B
	Mean for treatment	0.28 A	0.45 B	0.27 A	0.44 b	0.45 AB	3.45 C	

[1, 2] For explanations see Table 2.

[3] Means in the table followed by the same letter, for each sample separately, are not significantly different at a level α = 0.05, according to Duncan's multiple range test

Lettuce seeds of both samples stored at 20 °C were characterized by significantly lower fungal infestation and higher percentage of seeds free of fungi than those stored at 4 °C. In principle, treatment of seeds with acetic acid solutions significantly reduced the occurrence of all the fungi observed on seeds. The acetic acid solution at concentration of 2%, most effectively eliminated fungi, in many cases was more effective than fungicide (Tables 6).

Untreated seeds of the sample I after storage for 18 months were mostly occupied by *Alternaria alternata*, after storage at 4 °C this fungus was observed on 64.5% of seeds and after storage at 20 °C on 16.5%. Acetic acid at concentrations of 0.5 and 2% significantly decreased the seed settlement by *A. alternata*, and acid solution at concentration of 0.25% limited its infestation at fungicide level. All the applied methods of treating seeds with acetic acid reduced the percentage of seeds infected by *Fusarium* spp. compared to untreated seeds and seeds soaked in water. Untreated seeds in the sample I were occupied by *B. cinerea, M. simplex* and *S. botryosum* to a small extent, and the percentage of seeds possessed by these fungi did not exceed 3%. Acetic acid solutions reduced their occurrence at fungicide level or eliminated them completely. In the case of *Cladosporium* spp. the use of 0.25% acetic acid solution was ineffective. However, solutions with higher concentrations were more effective than the applied fungicide. With increasing concentration of acetic acid solution, the percentage of seeds free of fungi increased. The highest number of seeds free of fungi was observed after application of acetic acid solution of 2% concentration. Two times more seeds free of fungi were noted after storing seeds at 20 °C (Table 6).

Table 6. Seed health of untreated and treated lettuce seeds of samples I and II after 18 months of storage (%)

	Temperature [°C]	Seed treatment						Mean for temperature
		C[1]	W	F	0.25A	0.5A	2.0A	
		Sample I						
Alternaria alternata	4	64.5 f[2]	52.0ef	39.0 de	33.5 d	11.0 bc	10.0 bc	35.0 B
	20	16.5 c	6.5 b	8.0 ab	2.0 a	1.0 a	1.5 a	5.9 A
	Mean for treatment	40.5 D	29.3 C	23.5 BC	17.8 B	6.0 A	5.8 A	
Botrytis cinerea	4	2.5 c	0 a	0 a	1.0 b	0 a	0 a	0.6 B
	20	0 a	0 a	0 a	0 a	0 a	0 a	0 A
	Mean for treatment	1.3 B	0 A	0 A	0.5 AB	0 A	0 A	
Cladosporium spp.	4	32.5 d	15.5 c	18.0 c	30.5 d	2.0 ab	4.5 b	51.5 B
	20	1.5 ab	1.0 ab	0.5 a	2.5 ab	1.0 ab	1.0 ab	3.8 A
	Mean for treatment	17.0 C	8.3 B	9.3 B	16.5 C	1.5 A	2.8 A	
Fusarium spp.	4	49.0 e	65.5 f	7.5 bc	45.0 e	29.0 d	0 a	32.7 B
	20	12.5 c	11.0 c	0 a	3.5 b	4.0 b	0 a	15.5 A
	Mean for treatment	30.8 E	38.3 E	3.8 B	24.3 D	16.5 C	0 A	
Melanospora simplex	4	2.5 b	3.0 b	0 a	0.5 a	0 a	0 a	1.0 B
	20	0 a	0 a	0 a	0 a	0 a	0 a	0 A
	Mean for treatment	1.3 BC	1.5 C	0 A	0.3 AB	0 A	0 A	
Stemphylium botryosum	4	4.5 c	5.0 c	1.0 ab	2.5 bc	0.5 ab	0 a	2.3 B
	20	1.5 ab	1.0 ab	0 a	0 a	0 a	1.0 a	0.6 A
	Mean for treatment	3.0 B	3.0 B	0.5 A	1.3 A	0.3 A	0.5 A	
Seeds free of fungi	4	2.0 a	2.0 a	24.5 c	12. b	41.0 d	68.5 ef	25.0 A
	20	61.5 e	73.5 e-g	88.0 h	79.5 f-h	84.0 gh	87.5 h	57. 0 B
	Mean for treatment	31.8 A	37.8 A	56.3 C	45.8 B	62.5 C	78.0 D	

Sample II								
Alternaria alternata	4	99.0 h	99.0 gh	93.5 f	96.5 fg	24.0 c	1.0 a	68.8 B
	20	82.0 e	12.0 b	42.5 d	2.0 a	2.5 a	0.5 a	23.6 A
	Mean for treatment	90.5 F	55.5 D	68.0 E	49.3 C	13.3 B	0.8 A	
Botrytis cinerea	4	2.5 b	1.5 ab	5.5 c	11.5 d	0 a	0 a	3.5 B
	20	0 a	0 a	0 a	0 a	0 a	0 a	0 A
	Mean for treatment	1.3 BC	0.8 AB	2.8 CD	5.8 D	0 A	0 A	
Cladosporium spp.	4	7.5 d-f	5.0 c-e	9.5 ef	10.5 f	1.0 ab	1.0 ab	5.8 B
	20	3.0 bc	0 a	4.5 b-d	0 a	2.0 a-c	0 a	1.6 A
	Mean for treatment	5.3 C	2.5 AB	7.0 C	5.3 BC	1.5 A	0.5 A	
Fusarium spp.	4	28.0 ef	42.0 g	16.0 cd	35.5 fg	45.5 g	3.5 ab	28.4 B
	20	19.5 de	16.0 cd	4.0 ab	8.0 bc	7.0 b	1.0 a	9.3 A
	Mean for treatment	23.8 C	29.0 C	10.0 B	21.8 C	26.3 C	2.3 A	
Melanospora simplex	4	75.0 e	79.0 e	18.0 c	60.0 d	0 a	0 a	38.7 B
	20	21.0 c	1.5 b	2.0 b	0 a	0 a	0 a	4.1 A
	Mean for treatment	48.0 E	40.3 D	10.0 B	30.0 C	0 A	0 A	
Stemphylium botryosum	4	19.0 c	22.5 c	5.5 b	9.5 b	1.5 a	0.5 a	9.8 B
	20	1.5 a	5.0 b	1.0 a	0 a	0 a	0 a	1.3 A
	Mean for treatment	10.3 C	13.8 D	3.3 B	4.8 B	0.8 A	0.3 A	
Seeds free of fungi	4	0 a	0 a	1.5 a	0 a	24.5 c	86.5 fg	18.8 A
	20	8.5 b	65.0 e	45.0 d	79.5 f	79.0 f	91.0 g	61.3 B
	Mean for treatment	4.3 A	32.5 BC	23.3 B	39.8 C	51.8 D	88.8 E	

[1]For explanations see Table 1

[2]Means in the table followed by the same letter, for each sample separately, are not significantly different at a level α = 0.05, according to Duncan's multiple range test

Untreated seeds in sample II were most often infected with *A. alternata*, after storage at 4 °C 99% of seeds were occupied by this fungus and at higher temperature 82%. A solution of 0.25% acetic acid at 4 °C reduced the occurrence of *A. alternata* at fungicide level and was more effective at 20 °C. *Botrytis cinerea* was present on the seeds only after storing them at a lower temperature. The percentage of seeds infected by this fungus was small and was 2.5%. Infection of seeds by *B. cinerea* significantly increased after application of fungicide and 0.25% acetic acid. However, after application of higher acetic acid concentrations, that fungus was completely eliminated. The effect of acetic acid on the presence of fungi of the genus *Fusarium* was not unambiguous, the solution of the highest concentration limited their occurrence more effectively than the fungicide, 0.5% solution, similarly to soaking in water, stimulated their development and 0.25% did not significantly affect their occurrence. Acetic acid solutions with concentrations of 0.5 and 2% significantly reduced the occurrence of *Cladosporium* spp. and fungicide was ineffective. It was found also that these solutions, after storing the seeds, irrespective of temperature, completely eliminated *M. simplex*. This fungus was occupied the untreated seeds stored at 4 °C in 75% and in case of higher storing temperature in 21%. Soaking the seeds in distilled water resulted in an increase in their settlement by *Stemphylium botryosum,* while acetic acid proved effective in reducing it. Solutions with concentrations of 0.5 and 2% were more effective than fungicide. The percentage of seeds free of fungi was higher after storing the seeds at 20 °C (61.3%). Moreover, the higher concentration of acetic acid, the higher percentage of seeds free of fungi. All methods of seed treatment significantly improved this parameter in comparison with fungicide (Table 6).

Discussion

The organic acids treatment can successfully replace chemical treatment, weak organic acids are commonly used as preservative agents in food industry (Kang *et al.*, 2003). Acetic acid and other weak organic acids slow the growth of various spoilage bacteria, yeasts, and molds (van Beilen *et al.* 2014, Hassan *et al.* 2012, Hassan *et al.* 2015). It was found that acetic acid vapours at concentrations 8 $\mu l \bullet l^{-1}$ caused growth inhibition and spore germination of *Alternaria* sp., *Aspergillus flavus, A. niger, A. ochraceus, A. terreus, Fusarium miniliforne* and *Penicillium* spp. (Abd-Alla, 2005; Morsy *et al.*, 2000 a, b). Guimarães *et al.* (2018) showed that organic acids also inhibit mycotoxin production by fungi. Luz *et al.* (2021) recommends the use of peracetic acid, obtained from the acetylation reaction of acetic acid with hydrogen peroxide, vapour to protect cereals for human consumption against toxigenic fungi, as it can inhibit the production of aflatoxin B1 and ochratoxin.

It has been found in the present investigation that treating seeds with the acetic acid solution at concentration of 0.5% and stored at 4 and 20 °C did not affect negatively on seed germination at first and final counts and limited the incidence of fungi, especially *Alternaria alternata, Melanospora simplex* and *Stemphylium botryosum*. The effect of this solution was effective at the fungicide level, and in case of reduced occurrence of *A. alternata* better. Before storage, in the case of sample II, which had poorer germination, a significant improvement in germination was observed after treatment with acetic acid solutions at concentration of 0.25 and 0.5%. These solutions also effectively reduced the incidence of the previously mentioned fungi. This suggests that the elimination or reduction of fungi on seeds, improves the germination parameters of these seeds. Kang *et al.* (2003) investigated that weak organic acids, especially acetic acid, limited the growth of *Colletotrichum gleosporioides*. Authors observed an inhibition of respiration of fungus and stated that inhibitory might be more enhanced by undissociated form of acetic acid and effect might be exerted within the cell. Inhibition effect was not connected with cell damage of fungus. Kang *et al.* (2003) reported that organic acids decrease the pH value and acidifying the fungus cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis. As a consequence of the high energy consumption for this purpose, fungal growth will be limited. Wang *et al.* (2021) study an effect of weak organic acids on the growth of *Botrytis cinerea* determined for their activity. They observed that cuminic acid may inhibit the development of sclerotia and the exudation of oxalic acid. Moreover, authors found that cuminic acid could impact on the cell membrane integrity, and down regulate the expression of genes involved in sclerotia development and pathogenicity of that fungus. In present study acetic acid at higher concentrations effectively limited *B. cinerea*.

The storage temperature of the seeds was not negligible. Low temperatures and low seed moisture content are recommended for long-term storage of the orthodox seeds. These conditions are also favourable to the survival of the seed-borne fungi for a longer period. For both tested samples, significantly less fungi were observed on seeds stored at higher temperatures. Storage temperature also affected seed vigour and germination. After 18 months, significantly better values of these parameters were noted after storing seeds at 20 °C. Similar results were obtained by Dorna *et al.* (2021), carrot seeds treated with acetic acid and stored at 4 °C were characterized by a higher seed infestation with fungi than those stored at 20 °C. The effect of temperature on seed vigour was also confirmed by Brits *et al.* (2015), they found that low seed storage temperature of *Leucospermum cordifolium* Fourc. maintained high viability and vigour for about two years, while storage at room temperature led to a notable decline after only 1 year.

Using the highest concentration of acetic acid (2.0%), eliminated fungi from the seeds to the greatest extent. This phenomenon was observed both before and after seed storage. Szopińska (2013) also reported that acetic acid at higher concentrations (2.5 and 5.0%) effectively eliminated seed-borne fungi. However, at the same time, the author observed deterioration of seed germination and vigour. In the present experiment, negative effects on these parameters were observed before and after seed storage, especially after storage at higher temperature. Tunes *et al.* (2012) found no reduction in the germination capacity of winter wheat treated with acetic acid at concentrations of 0, 4, 8, 12, 16 mM, while they observed a significant shortening of seedling roots with increasing acid concentration. According to the authors, root shortening probably occurred because acetic acid affects the integrity of plasma membranes and interferes with energy production processes in the plant, such as oxidative phosphorylation and respiration. According to Rao and Mikkelsen (1977), the phytotoxic effects of acetic acid are closely related to the method of application and the concentration of acid. To reduce the phytotoxic effect, Dorna *et al.* (2018) recommend rinsing the seeds with water after treatment. In our study, despite rinsing the seeds, a significantly higher percentage of abnormal deformed seedlings was observed after application of 2.0% acetic acid. This percentage was also higher after seed storage, regardless of storage temperature.

Conclusions

The research showed that seed treatment of weak acetic acid solutions effectively reduced the incidence of fungi, especially *Alternaria alternata*, without limiting seed germination. Seeds stored at temperature 4 °C after soaking in weak acetic acid solutions were germinate at high level and were less occupied by fungi. Only treatment of seeds with 2.0% acetic acid solution negatively affected all seed quality parameters, indicating a phytotoxic effect.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The publication was co-financed within the framework of the Ministry of Science and Higher Education program as "Regional Initiative Excellence" in 2019–2022, Project No. 005/RID/2018/19, financing amount: 12,000,000 PLN.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abd-Alla MA (2005). Effect of acetic acid fumigation on common storage fungi of some medicinal and aromatic seeds. Egyptian Journal of Phytopathology 33(1):77-86.
Banwart GJ (1989). Basic Food Microbiology. Springer, Boston, MA

Brits GJ, Brown NAC, Calitz FJ, van Staden J (2015). Effects of storage under low temperature, room temperature and in the soil on viability and vigour of *Leucospermum cordifolium* (Proteaceae) seeds. South African Journal of Botany 97:1-8. *https://doi.org/10.1016/j.sajb.2014.11.003*

Dorna H, Yangyang QI, Szopińska D (2018). The effect of acetic acid, grapefruit extract and selected essential oils on germination, vigour and health of carrot (*Daucus carota* L.) seeds. Acta Scientiarum Polonorum Hortorum Cultus 17(2):27-38. *https://doi.org/10.24326/asphc.2018.2.3*

Dorna H, Rosińska A, Szopińska D (2021). The effect of acetic acid treatments on the quality of stored carrot (*Daucus carota* L.) seeds. Agronomy 2021(11): 1176. *https://doi.org/10.3390/agronomy11061176*

Guimarães A, Venancio A, Abrunhosa L (2018). Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum*. Food Additives and Contaminants: Part A 35(9):1803-1818. *https://doi.org/10.1080/19440049.2018.1500718*

Hassan R, El-Kadi S, Sand M (2015). Effect of some organic acids on some fungal growth and their toxins production. International Journal of Advances in Biology 2(1):1-11.

Hassan R, Sand M, El-Kadi S (2012). Effect of some organic acids on fungal growth and their toxins production. Journal of Agricultural Chemistry and Biotechnology 3(9):391-397. *https://doi.org/10.21608/JACB.2012.55011*

Kang HC, Park YH, Go SJ (2003). Growth inhibition of the phytopathogenic fungus, *Colletotrichum* species by acetic acid. Microbiological Research 158:321-326. *https://doi.org/10.1078/0944-5013-00211*

Ismaiel AA, Papenbrock J (2015). Mycotoxins: Producing fungi and mechanisms of phytotoxicity. Agriculture 5(3):492-537. *https://doi.org/10.3390/agriculture5030492*

ISTA (2020). International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.

Jalink H, van der Schoor R (1999). SeedCalculator 2.1. License Number: 100200122; Plant Research International: Wageningen, The Netherlands.

Khairnar DN, Kelhe AS, Khairnar AB (2011). Fungal diversity and mycotoxin effect on seed-borne fungi, seed germination and seedling vigour of some cereals of Nashik District. Nature Environment and Pollution Technology 10(3):485-486. *https://neptjournal.com/upload-images/NL-35-31-(31)B-1696.pdf*

Luz C, Carbonell R, Quiles JM, Torrijos R, de Melo Nazareth T, Mañes J, Mecca G (2021). Antifungal activity of peracetic acid against toxigenic fungal contaminants of maize and barley at the postharvest stage. LWT – Food Science and Technology 148:111754. *https://doi.org/10.1016/j.lwt.2021.111754*

Mancini V, Romanazzi G (2014). Seed treatments to control seedborne fungal pathogens of vegetable crops. Pest Management Science 70:860-868. *https://doi.org/10.1002/ps.3693*

Mathur SB, Kongsdal O (2003). Common laboratory seed health testing methods for detecting fungi. International Seed Testing Association. Bassersdorf, Switzerland.

Morsy AA, Abd-El-Kareem F, Abd-Alla MA (2000a). Effect of acetic acid fumigation on common storage fungi of some grains. Egyptian Journal of Phytopathology 28:95-106.

Morsy AA, Abd-Alla MA, Abd-El-Kareem F (2000b). Effect of acetic acid fumigation of wheat grains on fungal infection during storage. Egyptian Journal of Phytopathology 28:107-117.

Rao DN, Mikkelsen DS (1977). Effect of acetic, propionic, and butyric acids on young rice seedlings growth. Agronomy Journal 69:923-928. *https://doi.org/10.2134/agronj1977.00021962006900060008x*

Sholberg PL, Gaunce AP (1995). Fumigation of fruit with acetic acid to prevent postharvest decay. HortScience 30:1271-1275. *https://doi.org/10.21273/HORTSCI.30.6.1271*

Szopińska D (2013). The effect of organic acids treatment on germination, vigour and health of zinnia (*Zinnia elegans* Jacq.) seeds. Acta Scientiarum Polonorum Hortorum Cultus 12(5):17-29.

Szopińska D, Tylkowska K (2003). Effect of osmopriming on location of seed-borne fungi in lettuce (*Lactuca sativa*) seeds. Phytopatologia Polonica 29:69-80.

Tsedaley B (2015). Review on seed health tests and detection methods of seedborne diseases. Journal of Biology, Agriculture and Healthcare 5(5):176-184.

Tunes LM, Avelar SAG, Barros ACSA, Pedroso DC, Muniz MFB, Menezes NL (2012). Critical levels of organic acids on seed germination and seedling growth of wheat. Revista Brasileira de Sementes 34(3):366-372. *https://doi.org/10.1590/S0101-31222012000300002* van Beilen JWA, Teixeira de Mattos MJ, Hellingwerf KJ, Brul S (2014). Distinct effects of sorbic acid and acetic acid on the electrophysiology and metabolism of *Bacillus subtilis*. Applied and Environmental Microbiology 80(19):5918-5926. *https://doi.org/10.1128/AEM.01391-14*

van der Wolf JM, Bimbaum Y, van der Zouwen PS, Groot SPC (2008). Disinfection of vegetable seed by treatment with essential oils, organic acids and plant extracts. Seed Science and Technology 36:76-88. *https://doi.org/10.15258/sst.2008.36.1.08*

Wang Y, Qiao Y, Zhang M, Ma Z, Xue Y, Mi Q, Wang A, Feng J (2021). Potential value of small-molecule organic acids for the control of postharvest gray mold caused by *Botrytis cinerea*. Pesticide Biochemistry and Physiology 177:104884. *https://doi.org/10.1016/j.pestbp.2021.104884*

Watanabe T (2002). Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species. CRC PRESS, Boca Raton, London, New York, Washington.