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THE EFFECT OF HYDROGEN PEROXIDE ON SEED QUALITY AND EMERGENCE OF CARROT (*Daucus carota* L.)

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ABSTRACT

Infestation with pathogenic fungi is frequently a cause of poor carrot seed quality. The effect of hydrogen peroxide (H_2O_2) treatment on germination, vigour and health of carrot seeds as well as plant emergence was investigated. Four seed samples were tested. Seeds were soaked in 3, 6, 9 and 12% H_2O_2 solutions for 10, 30 and 60 min. Controls were untreated seeds, seeds treated with fungicide (a.i. 75% thiram), and seeds soaked in distilled water for 10, 30 and 60 min. The fungi from genera *Alternaria* and *Fusarium* were frequently detected on tested seeds and dead seedlings. Hydrogen peroxide treatment, regardless of concentration and treatment time, reduced significantly carrot seed infestation with *A. alternata*, *A. radicina* and *Fusarium* spp., however was not effective in the control of *A. dauci*. Deterioration of seed germination and vigour was observed mostly if seeds were treated with 9 and 12% H_2O_2. Treating seeds with 3% H_2O_2 for 30 min positively affected plant emergence in three of four tested samples, while treatment with 6% H_2O_2 significantly decreased percentage of healthy seedlings in one of these samples and increased it in two of them.

Key words: H_2O_2, fungi, carrot, seed germination, seed vigour, seedling emergence

INTRODUCTION

Carrot (*Daucus carota* L.) is one of the most popular vegetable on the world. According to FAO, Poland, with a yield over 742 thousand tons and production area exceeding 19 thousand hectares, belongs to the leading producers of carrot, following China, Uzbekistan, Russian Federation, United States and Ukraine [FAOSTAT 2016]. Seeds infected by *Alternaria* spp. had been shown in a number of countries from different geographical regions to be a major source of severe diseases of carrot, as well as a reason of low seed germination and seedling emergence. Among *Alternaria* species *A. radicina*, known mainly as casual agent of black rot of roots, and *A. dauci*, responsible for leaf blight, are regarded as the most important seed-borne fungal pathogens of carrot [Tylkowska 1992]. Moreover, Jarosz [2006] frequently detected on carrot seeds *Alternaria alternata* and *Epicoccum nigrum*, and numerous fungi from genera *Fusarium* and *Penicillium*. Among them *A. alternata* is considered by some researchers as weak pathogen of carrot, which may negatively affect seed germination [Tylkowska 1991]. Chemical compounds have been commonly used to control health of the seeds. However, there is a global need for a more organic production, including discarding standard chemical crop protectants [Groot et al. 2004]. Hydrogen perox-

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ide (H_2O_2), produced naturally in plant cells under stressful conditions, is characterized by high oxidative reactivity [Ogawa and Iwabuchi 2001]. Because of antibacterial and antifungal properties, H_2O_2 is commonly use in medicine and food industry for disinfection of various products and surfaces [Lehrer 1969, Thomas 1979, Sapers and Sites 2003]. There are many reports that hydrogen peroxide treatment may improve seed germination. This phenomenon has been observed in several plants, such as: barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) [Smilanick et al. 1994], camphor tree (*Cinnamomum camphora*) [Chien and Lin 1994], eastern gamagrass (*Tripsacum dactyloides*) [Klein et al. 2008], maize (*Zea mays*) [Wahid et al. 2008], muscadine (*Vitis rotundifolia*) [Conner 2008], rice (*Oryza sativa*) [Naredo et al. 1998, Sasaki et al. 2005], watermelon (*Citrullus lanatus*) [Duval and NeSmith 2000, Jaskani et al. 2006], and zinnia (*Zinnia elegans*) [Ogawa and Iwabuchi 2001]. According to Klein et al. [2008] and Ogawa and Iwabuchi [2001] promotion of germination is probably caused by oxidation of inhibitors presented in the pericarp by H_2O_2, or a direct physical scarifying effect of this compound, or both. For the improvement of seed germination and plant emergence might be also responsible antibacterial and antifungal activity of H_2O_2. Hydrogen peroxide treatment effectively controlled *Sclerospora graminicola* on pearl millet [Geetha and Shetty 2002], seed-borne fungi from genus *Fusarium* on conifer seeds [Neumann et al. 1997], *Peronospora tabacina* sporulation on tobacco leaf disks [Peng and Kuc 1992], germination of *Tilletia controversa* and *T. tritici* teliospores contaminating wheat grains [Smilanick et al. 1994], and seed-borne fungi *Alternaria zinniae* and *Fusarium* spp. on zinnia seeds [Szopińska 2014]. Preliminary experiments showed that hydrogen peroxide may also reduce infestation of carrot seeds with seed-borne fungi from genus *Alternaria* [Jarosz 2006]. However, further studies are needed to determine optimal conditions of carrot seed treatment with this compound. Therefore, this experiment was performed to investigate the effect of hydrogen peroxide treatment on the health, germination and vigour of carrot seeds as well as plant emergence.

MATERIALS AND METHODS

Four commercially produced carrot seed samples, cv. Amsterdam (samples I and II), cv. Flakkese (sample III) and cv. Perfekcja (sample IV), obtained from TORSEED Seed Company in Toruń, were used in the experiment. Hydrogen peroxide (30% solution) obtained from Sigma-Aldrich Co. was used for seed treatment. Moreover, fungicide Zaprawa Nasienna T 75 WS/DS (a.i. 75% thiram), produced by Organika-Azot in Jaworzno, was applied as an alternative chemical control.

Seed treatment

Seed health, germination and vigour tests were performed for: seeds soaked in 3, 6, 9 and 12% H_2O_2 solutions for 10, 30 and 60 min, untreated seeds (control), seeds treated with Zaprawa Nasienna T 75 WS/DS at a dose 5 g per 1 kg of seeds (chemical control), and seeds soaked in distilled water for 10, 30 and 60 min (water control). Additionally, seedling emergence test was performed for: untreated seeds, and seeds treated with 3 and 6% H_2O_2 solutions for 30 min. These combinations were chosen on the base of the results of health, germination and vigour tests.

Mycological analysis

Deep-freeze blotter test was performed on five replications of 40 seeds from each treatment according to ISTA rules [International Rules for Seed Testing 2014 a, b]. 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 [Machado et al. 2002, Mathur and Kongsdal 2003]. Additionally the percentage of seeds free of fungi was evaluated.

Seed germination and vigour tests

For germination and vigour tests separately six replications of 50 seeds from each treatment were

incubated in 9 cm diameter Petri dishes on six layers of filter paper, 50 seeds per dish, in darkness at 20°C. Percentage of normal seedlings (germination capacity) was determined after seven and fourteen days according to the ISTA rules [International Rules for Seed Testing 2012]. The total number of germinating seeds (G_{max}) as well as germination rates, characterizing seed vigour i.e.: T_{10} – time to 10% of G_{max}, and T_{50} – time to 50% of G_{max} were determined on the base of number of seeds with visible radicle counted daily.

Seedling emergence test

The test was performed in multi-cell trays (3 × 4 cm cell, 103 cells per tray) with universal pot soil (Kronen) moistened with distilled water. For each treatment four replications of 25 seeds were tested. Seeds were incubated for 20 days at 20°C, under 12 h alternating cycles of day light and darkness. Seedlings emergence and health were assessed after 6, 8, 10, 12, 14, 16, 18 and 20 days. Number of healthy emerged seedlings was evaluated after 20 days. Dead seedlings were removed successively from multi-cell trays, washed with distilled water and incubated in 9 cm diameter Petri dishes on six layers of filter paper for 7 days at 20°C, under 12 h alternating cycles of NUV light and darkness. After incubation the fungi growing on seedlings were identified on the basis of their growth and sporulation using a stereomicroscope [Machado et al. 2002, Mathur and Kongsdal 2003].

Data analysis

SeedCalculator version 2.1 software [Jalink and van der Schoor 1999] was applied to analyze G_{max} and vigour data. All results were compared by means of variance analysis followed by the Duncan's test.

RESULTS

Mycological analysis

Soaking seeds in water did not influence the percentage of seeds free of fungi in tested samples (tab. 1). However, if hydrogen peroxide was applied, regardless of concentration and treatment time, significant increase in the number of seeds free of fungi was detected in samples II, III and IV. In sample I, this effect was observed in seeds treated with 3% H_2O_2 for 60 min, 6% H_2O_2 for 30 and 60 min and 9 and 12% H_2O_2, regardless of treatment time. In samples I and IV the effectiveness of hydrogen peroxide treatment was even higher than fungicide application, especially if the highest concentrations of H_2O_2 were used for 60 min. Pathogenic *Alternaria* species, i.e.: *A. dauci* and *A. radicina*, were detected in all the samples, however *A. radicina* prevailed (tab. 2). *Alternaria dauci* infected 7, 2.5, and 6% of untreated seeds of samples I, II and IV, respectively, and was not found in control seeds of sample III. Low level of seed infection with this pathogen was observed only when seeds of this sample were soaked in distilled water for 10 and 30 min. Applied treatments affected growth of *A. dauci* in sample IV, in which, decrease in seed infection with this fungus was found after treatment with 6% H_2O_2 for 60 min, 9% H_2O_2 for 10 and 60 min, and 12% H_2O_2 for 10, 30 and 60 min. *Alternaria radicina* was detected in 55, 26, 7 and 11% of untreated seeds of samples I, II, III and IV, respectively. After soaking seeds in water a decrease in seed infection with this pathogen in relation to untreated seeds was detected only in sample III. Hydrogen peroxide treatment, regardless of concentration and time, effectively reduced the percentage of seeds infected by *A. radicina* in all samples. Moreover, in sample I, this treatment was many a time more effective than applied fungicide. Positive effect of hydrogen peroxide treatment was recorded also in relation to other fungi detected on tested seeds. Among them *A. alternata* and fungi from genus *Fusarium* prevailed (tab. 3). *Alternaria alternata* was found on 96, 98, 88 and 100% of untreated seeds of sample I, II, III and IV, respectively. Soaking seeds in water resulted in an increase in their infestation with this fungus in samples I and II, while in sample III decrease in seed infestation was observed. Nevertheless, applied hydrogen peroxide treatment, regardless of concentration and time, limited number of seeds infested with *A. alternata*, in relation to untreated seeds and water control, in all samples. The same phenomenon was observed in case of seed infestation with *Fusarium* spp. in samples I, II and IV.

On seeds of sample III fungi from this genus were recorded sporadically, and applied treatment usually did not affect their incidence.

Seed germination and vigour

Applied treatments did not affect total seed germination in samples I, II and IV (tab. 4). The decrease in the number of germinating seeds (G_{max}) was observed only in sample III, if seeds were soaked in 9% H_2O_2 solution for 30 and 60 min and in 12% H_2O_2 solution for 10, 30 and 60 min. Hydrogen peroxide applied at 3 and 6% concentration did not influence germination capacity in all samples. However, significant decrease in this parameter was observed in sample I after treating seeds with 9% H_2O_2 solution for 10 and 30 min, in sample III if seeds were treated in 9 and 12% H_2O_2 solutions for 60 min and in 12% H_2O_2 solution for 30, and in sample IV if seeds were soaked in 9% H_2O_2 solution for 10 and 30 min, as well as in 12% H_2O_2 solution, regardless of treatment time.

Table 1. The effect of hydrogen peroxide treatment on the percentage of carrot seeds free of fungi

Treatment	Treatment time (min)	Sample I (cv. Amsterdam)	Sample II (cv. Amsterdam)	Sample III (cv. Flakkese)	Sample IV (cv. Perfekcja)
control[1]	–	0 a[5]	0 a	10.5 a	0 a
fungicide[2]	–	10.0 de	25.0 f	99.5 e	34.5 cd
distilled water[3]	10	0 a	0 a	19.0 ab	0 a
	30	0 a	0 a	18.5 ab	0 a
	60	0 a	0 a	26.5 b	0 a
3% H_2O_2[4]	10	0 a	2.5 b	58.0 c	20.5 b
	30	1.5 ab	8.5 cd	78.5 d	33.0 c
	60	3.0 bc	9.0 cd	79.5 d	32.5 c
6% H_2O_2	10	1.5 ab	5.0 bc	74.5 d	40.0 c–e
	30	10.0 de	7.5 cd	83.5 d	38.5 c–e
	60	11.0 de	12.0 d	63.5 c	61.0 f
9% H_2O_2	10	5.0 cd	8.5 cd	78.0 d	44.0 de
	30	6.0 cd	10.0 cd	80.5 d	45.5 e
	60	21.0 f	13.0 d	64.0 c	60.5 f
12% H_2O_2	10	8.0 de	14.5 de	62.0 c	47.0 e
	30	14.5 ef	21.5 ef	64.0 c	47.0 e
	60	33.0 g	23.0 ef	27.0 b	63.0 f

[1] Control – untreated seeds
[2] Seeds treated with Zaprawa Nasienna T 75 WS/DS at a dose 5 g per 1 kg of seeds
[3] Seeds soaked in distilled water
[4] Seeds soaked in 3, 6, 9 and 12% solution of hydrogen peroxide, respectively
[5] Means in columns followed by the same letters are not significantly different according to the Duncan's test at the level $\alpha = 0.05$

Table 2. The effect of hydrogen peroxide treatment on the percentage of carrot seeds infected by *Alternaria dauci* and *A. radicina*

	Treatment	Treatment time (min)	Sample I (cv. Amsterdam)	Sample II (cv. Amsterdam)	Sample III (cv. Flakkese)	Sample IV (cv. Perfekcja)
Alternaria dauci	control	–	7.0 a	2.5 a–d	0 a	6.0 d
	fungicide	–	3.5 a	0.5 a	0 a	2.5 b–d
	distilled water	10	9.5 a	3.5 a–d	0.5 b	6.0 d
		30	10.0 a	2.0 a–d	0.5 b	4.0 cd
		60	6.0 a	1.5 a–c	0 a	4.5 d
	3% H_2O_2	10	5.5 a	5.0 cd	0 a	2.5 bd
		30	8.5 a	6.0 d	0 a	3.5 cd
		60	9.0 a	3.0 a–d	0 a	6.5 d
	6% H_2O_2	10	8.5 a	1.0 ab	0 a	2.5 b–d
		30	4.5 a	2.0 a–d	0 a	3.5 cd
		60	4.5 a	5.0 b–d	0 a	1.5 a–c
	9% H_2O_2	10	4.5 a	1.0 ab	0 a	0 a
		30	6.5 a	1.0 ab	0 a	2.5 b–d
		60	6.5 a	1.5 a–d	0 a	0.5 ab
	12% H_2O_2	10	3.5 a	2.5 a–d	0 a	0.5 ab
		30	7.0 a	2.0 a–d	0 a	0.5 ab
		60	6.0 a	1.5 ab	0 a	0.5 ab
Alternaria radicina	control	–	55.0 de	26.0 d	7.0 c	11.0 c
	fungicide	–	42.5 cd	5.0 bc	0 a	2.5 ab
	distilled water	10	55.0 de	20.5 d	2.5 b	6.5 bc
		30	60.0 e	23.5 d	3.5 b	9.0 c
		60	67.5 e	19.5 d	4.0 b	6.0 bc
	3% H_2O_2	10	40.5 c	8.0 c	0 a	1.0 a
		30	30.0 bc	3.0 a–c	0 a	1.0 a
		60	23.5 b	4.0 a–c	0 a	3.0 ab
	6% H_2O_2	10	23.0 b	5.0 bc	0 a	0 a
		30	17.0 ab	2.0 a–c	0 a	1.0 a
		60	18.5 ab	2.5 ab	0 a	1.5 a
	9% H_2O_2	10	26.0 b	1.0 a	0 a	1.5 a
		30	23.5 b	3.0 a–c	0.5 a	2.0 a
		60	18.0 ab	2.0 a–c	0 a	2.0 a
	12% H_2O_2	10	18.5 ab	1.0 a	0 a	0.5 a
		30	21.0 ab	1.5 ab	0 a	1.5 a
		60	10.5 a	3.5 a–c	0.5 a	0.5 a

For explanations see table 1

Table 3. The effect of hydrogen peroxide treatment on the percentage of carrot seeds infested with *Alternaria alternata* and *Fusarium* spp.

	Treatment	Treatment time (min)	Sample I (cv. Amsterdam)	Sample II (cv. Amsterdam)	Sample III (cv. Flakkese)	Sample IV (cv. Perfekcja)
Alternaria alternata	control	–	96.0 f	98.0 g	88.0 f	100.0 g
	fungicide	–	65.5 a–d	71.0 bc	0.5 a	52.5 de
	distilled water	10	100.0 g	100.0 h	75.5 e	100.0 g
		30	100.0 g	100.0 h	75.0 e	98.5 g
		60	100.0 g	100.0 h	67.0 e	99.0 g
	3% H_2O_2	10	82.5 e	90.0 f	10.5 bc	70.0 f
		30	74.5 c–e	78.5 c–e	4.0 b	60.0 e
		60	77.5 de	84.0 d–f	4.5 b	47.0 cd
	6% H_2O_2	10	69.0 cd	79.0 ef	14.0 cd	48.5 cd
		30	72.5 c–e	87.5 d–f	11.5 cd	52.5 de
		60	70.0 cb	80.0 c–e	15.5 cd	35.0 ab
	9% H_2O_2	10	67.0 b–d	84.5 d–f	9.0 bc	51.0 de
		30	75.5 de	80.0 c–e	9.0 bc	45.5 b–d
		60	53.0 a	79.0 c–e	14.5 cd	30.0 a
	12% H_2O_2	10	75.5 de	78.5 cd	18.0 cd	38.0 a–c
		30	61.5 ac	66.0 ab	13.0 cd	45.0 b–d
		60	55.0 ab	57.5 a	22.5 d	30.0 a
Fusarium spp.	control	–	60.5 fg	17.5 d	0 a	15.0 d
	fungicide	–	25.5 de	2.5 ab	0 a	3.5 a–c
	distilled water	10	70.5 g	9.5 c	0 a	27.0 e
		30	67.0 g	21.5 d	0 a	37.5 e
		60	52.5 f	16.0 d	1.5 b	15.0 d
	3% H_2O_2	10	20.5 cd	4.0 bc	0.5 a	3.0 a–c
		30	27.0 de	1.5 ab	0 a	3.0 a–c
		60	15.0 a–c	2.0 ab	0 a	2.0 a–c
	6% H_2O_2	10	34.0 e	1.5 ab	0 a	3.0 a–c
		30	15.0 a–c	1.5 ab	0 a	3.0 a–c
		60	20.0 cd	2.0 ab	0 a	0.5 a
	9% H_2O_2	10	27.5 de	3.5 bc	0 a	6.0 c
		30	16.0 a–c	0.5 a	0.5 a	3.5 a–c
		60	10.0 ab	0.5 a	0 a	4.0 a–c
	12% H_2O_2	10	18.0 b–d	2.5 ab	0.5 a	6.5 bc
		30	9.5 a	1.0 ab	0 a	3.5 a–c
		60	12.0 a–c	3.0 a–c	0.5 a	1.0 ab

For explanations see table 1

Table 4. The effect of hydrogen peroxide treatment on germination of carrot seeds

	Treatment	Treatment time (min)	Sample I (cv. Amsterdam)	Sample II (cv. Amsterdam)	Sample III (cv. Flakkese)	Sample IV (cv. Perfekcja)
Total seed germination – G_{max} (%)	control	–	86.7 a–c	90.7 ab	71.0 d–f	82.3 ab
	fungicide	–	85.3 a–c	93.0 b	75.3 f	86.3 ab
	distilled water	10	80.0 a	88.7 ab	66.7 c–e	84.3 ab
		30	89.0 c	90.3 ab	70.7 d–f	85.7 ab
		60	87.0 a–c	93.3 b	70.3 d–f	88.3 b
	3% H_2O_2	10	87.3 a–c	86.3 a	75.3 f	87.3 b
		30	84.7 a–c	88.3 ab	71.3 d–f	82.3 ab
		60	88.3 bc	88.0 ab	73.0 ef	81.0 ab
	6% H_2O_2	10	88.7 bc	90.0 ab	71.3 d–f	82.7 ab
		30	85.0 a–c	88.7 ab	65.3 c–e	84.3 ab
		60	84.3 a–c	91.0 ab	66.3 c–e	76.3 a
	9% H_2O_2	10	83.7 a–c	91.0 ab	63.7 cd	79.3 ab
		30	84.7 a–c	89.3 ab	61.3 bc	80.7 ab
		60	82.7 a–c	91.3 ab	55.0 ab	76.3 a
	12% H_2O_2	10	88.0 bc	86.3 a	60.0 bc	81.7 ab
		30	82.0 a–c	87.0 a	53.7 ab	78.7 ab
		60	81.3 ab	85.3 a	47.7 a	82.3 ab
Germination capacity (%)	control	–	70.0 de	67.0 a	62.3 cd	76.7 g
	fungicide	–	75.3 e	77.7 bc	62.3 cd	68.0 a–g
	distilled water	10	61.7 a–d	73.0 a–c	60.0 cd	74.3 e–g
		30	62.3 a–d	72.3 a–c	64.7 cd	75.7 e–g
		60	64.3 a–d	68.7 ab	62.0 cd	71.3 b–g
	3% H_2O_2	10	65.3 b–e	77.3 a–c	61.3 cd	73.3 e–g
		30	67.3 b–e	74.7 a–c	61.0 cd	72.0 c–g
		60	68.0 c–e	76.7 a–c	66.0 d	76.7 fg
	6% H_2O_2	10	62.3 a–d	80.0 c	61.7 cd	74.0 d–g
		30	61.7 a–d	74.0 a–c	56.0 b–d	68.7 a–g
		60	65.3 b–e	71.3 a–c	64.0 cd	71.3 b–g
	9% H_2O_2	10	57.3 a–c	73.3 a–c	60.7 cd	63.3 a–d
		30	53.3 a	74.0 a–c	54.3 bc	53.0 a–c
		60	59.7 a–d	76.7 a–c	46.3 ab	68.7 a–g
	12% H_2O_2	10	65.7 b–e	69.3 ab	55.0 bc	61.0 ab
		30	63.7 a–d	69.7 ab	41.3 a	58.0 a
		60	69.7 de	69.7 a–c	39.3 a	66.3 a–e

For explanations see table 1

Table 5. The effect of hydrogen peroxide treatment on the speed of carrot seed germination expressed by T_{10} and T_{50} parameters – time to 10 and 50% of total seed germination, respectively (days)

	Treatment	Treatment time (min)	Sample I (cv. Amsterdam)	Sample II (cv. Amsterdam)	Sample III (cv. Flakkese)	Sample IV (cv. Perfekcja)
T_{10}	control	–	1.67 c–e	2.36 c–f	2.58 c–e	2.10 c–e
	fungicide	–	1.86 ef	2.57 gh	2.60 c–e	2.44 f–h
	distilled water	10	1.10 a	2.36 c–f	2.35 a–c	1.81 bc
		30	1.41 bc	2.23 a–c	2.23 ab	1.97 cd
		60	1.25 ab	2.15 ab	2.25 ab	1.81 bc
	3% H_2O_2	10	1.48 bc	2.25 b–d	2.12 a	1.66 ab
		30	1.53 c	2.08 a	2.25 ab	1.49 a
		60	1.58 cd	2.16 ab	2.32 a–c	1.51 a
	6% H_2O_2	10	1.94 ef	2.42 d–g	2.46 b–d	2.15 d–f
		30	1.81 d–f	2.45 e–g	2.41 a–d	2.14 d–f
		60	1.94 ef	2.37 c–f	2.42 a–d	2.18 d–g
	9% H_2O_2	10	1.87 ef	2.34 c–f	2.60 c–e	2.21 d–h
		30	1.90 ef	2.35 c–f	2.71 de	2.22 d–h
		60	2.08 f	2.29 b–e	2.67 de	2.36 e–h
	12% H_2O_2	10	1.52 c	2.41 d–g	2.66 de	2.31 e–h
		30	1.93 ef	2.67 h	2.79 e	2.47 gh
		60	1.94 ef	2.51 fg	2.81 e	2.50 h
T_{50}	control	–	2.60 bc	3.16 a	3.70 bc	3.46 b–e
	fungicide	–	2.83 de	3.35 a–c	3.71 bc	3.81 ef
	distilled water	10	2.54 bc	3.26 ab	3.59 a–c	3.41 b–e
		30	2.45 a–c	3.18 a	3.35 ab	3.26 bc
		60	2.49 bc	3.25 ab	3.35 ab	3.25 bc
	3% H_2O_2	10	2.39 ab	3.24 ab	3.23 a	2.85 a
		30	2.23 a	3.13 a	3.30 ab	3.22 a–c
		60	2.26 a	3.22 a	3.59 a–c	3.20 ab
	6% H_2O_2	10	2.44 ab	3.26 ab	3.42 ab	3.69 d–f
		30	2.56 bc	3.27 ab	3.61 a–c	3.41 b–e
		60	2.58 bc	3.34 a–c	3.89 cd	3.54 b–e
	9% H_2O_2	10	2.69 cd	3.32 a–c	4.21 de	3.37 b–d
		30	2.59 bc	3.38 a–c	4.35 e	3.64 c–e
		60	3.00 e	3.56 cd	4.56 e	3.82 ef
	12% H_2O_2	10	2.45 a–c	3.48 bc	4.21 de	3.54 b–e
		30	2.93 e	3.76 de	4.42 e	3.80 ef
		60	2.97 e	3.87 e	4.60 e	4.06 f

For explanations see table 1

Table 6. The effect of hydrogen peroxide treatment on emergence of carrot seedlings (%)

	Treatment	Total emergence	Final healthy plant stand
Sample I (cv. Amsterdam)	control[1]	81.0 a[3]	48.0 a
	3% H_2O_2 30 min[2]	75.0 a	65.0 b
	6% H_2O_2 30 min	70.0 a	63.0 b
Sample II (cv. Amsterdam)	control	91.0 a	72.0 a
	3% H_2O_2 30 min	90.0 a	80.0 b
	6% H_2O_2 30 min	92.0 a	88.0 c
Sample III (cv. Flakkese)	control	60.0 a	53.0 a
	3% H_2O_2 30 min	56.0 a	52.0 a
	6% H_2O_2 30 min	61.0 a	54.0 a
Sample IV (cv. Perfekcja)	control	77.0 b	64.0 b
	3% H_2O_2 30 min	77.0 b	75.0 c
	6% H_2O_2 30 min	55.0 a	50.0 a

[1] Control – untreated seeds
[2] Seeds soaked for 30 min in 3 and 6% solution of hydrogen peroxide, respectively
[3] Means in columns followed by the same letters, for each sample separately, are not significantly different according to the Duncan's test at the level $\alpha = 0.05$

Deterioration of seed vigour parameters was observed only if seeds of tested samples were treated with hydrogen peroxide at 9 and 12% concentration (tab. 5). Prolongation of time needed to 10% of total seed germination (T_{10}) was noticed if seeds of sample I were treated with 9% H_2O_2 for 60 min. Higher concentration of H_2O_2 negatively affected T_{10} parameter in sample I and IV, if seeds were treated for 30 min, and 30 and 60 min, respectively. Deterioration of T_{50} parameter was recorded in sample I – after treatment with 9% H_2O_2 for 60 min and 12% H_2O_2 for 30 and 60 min, in sample II – if seeds were treated with 9% H_2O_2 for 60 min and 12% H_2O_2 for 10, 30 and 60 min, in sample III – in seeds treated with 9 and 12% H_2O_2, regardless of treatment time, and in sample IV – if seeds were soaked in 12% H_2O_2 solution for 60 min. In several cases, hydrogen peroxide at 3% concentration, as well as soaking seeds in water, positively affected seed vigour. Decrease in the value of T_{10} parameter was observed in sample I – if seeds were soaked in distilled water for 10 and 60 min, in sample II – in case of seeds soaked in distilled water for 60 min and in 3% H_2O_2 for 30 and 60 min, in sample III – if seeds were soaked in distilled water for 30 and 60 min and in 3% H_2O_2 for 10 and 30 min, and in seeds of sample IV treated with 3% H_2O_2, regardless of time. Time needed to 50% of total seed germination (T_{50}) was reduced only in sample I – if

seeds were treated with 3% H_2O_2 for 30 and 60 min, and in samples III and IV – after soaking seeds in 3% H_2O_2 solution for 10 min.

Seedling emergence test

Applied treatments did not affect total plant emergence in samples I, II and III (tab. 6). In sample IV, after seed treatment with 6% H_2O_2, the percentage of emerged seedlings decreased significantly, which subsequently negatively influenced final healthy plant stand. Treatment with 3% H_2O_2, on the other hand, increased the percentage of healthy plants in this sample as well as in samples I and II. Hydrogen peroxide at higher concentration significantly increased the healthy plant stand in samples I and II. None of applied treatments affected the percentage of healthy plants in sample III. The fungi from the genera *Alternaria* and *Fusarium* prevailed on the dead seedlings, regardless of treatment. *Alternaria radicina* was observed in all samples and infected 75, 60, 40 and 45% of dead seedlings in samples I, II, III and IV, respectively. *Alternaria dauci* was found on 20, 30 and 55% of these seedlings in samples I, II and IV, respectively. *Alternaria alternata* was accompanied usually by *Fusarium* spp., and these fungi were identified on 5, 10 and 60% of dead seedlings in samples I, II and III, respectively (data not shown).

DISCUSSION

Hydrogen peroxide treatment, regardless of concentration and treatment time, reduced significantly carrot seed infestation with fungi, especially *A. alternata*, *A. radicina* and *Fusarium* spp. Moreover, many a time H_2O_2 treatment was more effective than standard fungicide. Similar results observed Jarosz [2006] after treatment of carrot seeds with 3% H_2O_2 for 10, 20 and 30 min. The author reported, that applied treatments reduced seed infestation with *A. alternata*, *A. dauci*, *A. radicina*, and *Fusarium* spp., as well as positively affected seed germination. This beneficial effect was observed also in respect to the other species. Neumann et al. [1997] found, that soaking seeds of conifer plants in 3% H_2O_2 limited incidence of pathogenic *Fusarium* species on the seeds. Rosada [2012] and Słupinska [2012] reported that treatment of China aster and onion seeds, respectively, with 3% H_2O_2, resulted in an increase in the number of seeds free form fungi and reduction of their infestation with *A. alternata* and *Fusarium* spp. Szopińska [2014] found that hydrogen peroxide treatment caused a significant decrease in zinnia seed infestation with *A. alternata*, *A. zinniae* and *Fusarium* spp.

In present experiment tested samples varied in seed infestation with fungi, whereas seed-borne pathogenic species *A. dauci* and *A. radicina* were recorded in all of the samples. The negative effect of these fungi on plant emergence was observed especially in the samples characterized with high level of seed infection, for instance in sample I, in which 7 and 55% seeds were infected by *A. dauci* and *A. radicina*, respectively. The difference between total emergence and final healthy plant stand reached 33% for control in this sample. Hydrogen peroxide treatment lower this value to 10% if seeds were treated with 3% H_2O_2 and 7% if higher concentration of H_2O_2 was applied. The same phenomenon was observed also in sample II as regards to both variants of treatment, and in sample IV when 3% H_2O_2 solution was used. According to Tylkowska [1992] the increase in the seed infestation with *A. radicina* by 1% resulted in reduction of germination capacity by 0.3%. Hydrogen peroxide treatment significantly influenced seed infestation with this fungus, however, on the contrary to earlier observation of Jarosz [2006], was less effective against *A. dauci*. Therefore, this pathogen, presented on tested seed samples in a relatively low degree, had been frequently identified on dead seedlings in emergence test. Tylkowska [1991] reported, a decrease in germination capacity and an increase in the percentage of diseased seedlings after inoculation of disinfected carrot seeds with *A. alternata*. This potentially pathogenic fungus prevailed on the seeds of all tested samples and was also detected on dead seedlings of sample III in emergence test. However, *A. alternata* was often accompanied by fungi from genus *Fusarium* in this test, therefore, is difficult to conclude which fungus or fungi, or may-

be both, were responsible in this case for seedling damping-off. On the other hand, the frequent presence of *Fusarium* spp. on dead seedlings of sample III was not related with the results of seed health test, in which these fungi were detected on the seeds sporadically. There was probably caused by abundant growth of *A. alternata*, which overgrown other, less expansive fungi. In samples I, II and IV, the pathogenic properties of *A. alternata* were suppressed by activity of *A. dauci* and *A. radicina*, which were in most cases responsible for seedling damping-off in these samples. Generally, sample III seeds characterized the lowest seed quality, expressed by low total seed germination and germination capacity as well as low seed vigour. Seeds of this sample were infested mostly with *A. alternata*, which growth was significantly reduced on the seeds during H_2O_2 treatment. However, prolongation of treatment time (30, 60 min), especially if 6 and 9% solutions of hydrogen peroxide were used, resulted in further decrease in seed germination and vigour. On the other hand, total plant emergence and final healthy plant stand in this sample did not differ significantly in control and after H_2O_2 treatment. The results of previous experiments of Szopińska [2014] showed that the concentration of hydrogen peroxide in the higher extent than treatment time affected germination, vigour and health of zinnia seeds. In present study generally the same phenomenon was observed, however, in some cases, prolongation of treatment time over 10 or 30 min caused significant decrease in seed infestation with fungi but negatively affected seed germination and vigour, especially if higher, 9 and 12% concentrations of H_2O_2 were used.

There are many reports about promotion of seed germination by hydrogen peroxide treatment [Duval and NeSmith 2000, Ogawa and Iwabuchi 2001, Conner 2008, Klein et al. 2008, Huarte and Garcia 2009]. Narimanov [2000] reported that short-term H_2O_2 treatment promoted germination of barley, carrot, garden radish, haricot, maize, melon and vegetable marrow seeds and enhanced plant development. However, there are reports that higher concentrations of H_2O_2 can negatively affect germination parameters. Sasaki et al. [2005] found that soaking seeds of rice in 5, 50 and 100 mM H_2O_2 (ca. 0.016, 0.15 and 0.31%) solutions for 24 h significantly increased the shoot fresh weight of the seedlings, but treatment with 500 and 1000 mM H_2O_2 (ca. 1.5 and 3.1%) reduced this value.

Because, treating seeds of some samples with 9 and 12% H_2O_2 resulted in deterioration of seed germination and vigour, emergence test was performed only for seeds treated with 3 and 6% H_2O_2. Applied treatments generally did not affect total plant emergence, with the exception of sample IV in which treating seeds with 6% H_2O_2 resulted in significant decrease in this parameter followed by low final healthy plant stand.

CONCLUSIONS

1. Hydrogen peroxide treatment, regardless of concentration and treatment time, reduced significantly carrot seed infestation with fungi, especially *A. alternata*, *A. radicina* and *Fusarium* spp.

2. Deterioration of seed germination and vigour was observed mostly if seeds of tested samples were treated with hydrogen peroxide at 9 and 12% concentration.

3. Treating seeds with 3% H_2O_2 for 30 min positively affected plant emergence in three of four tested samples, whereas if seeds were treated with 6% H_2O_2 a significant decrease in the percentage of emerged seedlings was observed in one of these samples.

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