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THE EFFECT OF ACETIC ACID, GRAPEFRUIT EXTRACT AND SELECTED ESSENTIAL OILS ON GERMINATION, VIGOUR AND HEALTH OF CARROT (*Daucus carota* L.) SEEDS

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ABSTRACT

Seed infection with *Alternaria* spp. is an important source of severe carrot diseases. The aim of the study was to evaluate the effects of carrot seed treatments with acetic acid, grapefruit extract and volatile compounds of fir (*Abies alba* Mill.) and thyme (*Thymus vulgaris* L.) essential oils on their germination, vigour and health. Seeds of two samples were soaked for 30 min in 0.5, 1 and 2% acetic acid, 0.5 and 1% Biosept Active (33% of grapefruit extract), or treated for 72 h or 96 h with volatile compounds of fir and thyme oils individually (10 µl), and jointly (5+5 µl). Controls were untreated seeds and seeds soaked in distilled water. Acetic acid effectively controlled *A. alternata* and *A. radicina* in both samples, and did not affect adversely seed germination, however, at the highest concentration deteriorated seed vigour. Biosept Active was less effective, however at 1% concentration decreased seeds infestation with *A. alternata* in sample I, and at concentrations of 0.5 and 1% reduced percentages of seeds infested with *A. alternata* and *A. radicina* in sample II. Essential oils treatments in some cases favoured growth of *A. dauci* and *A. radicina*.

Key words: *Alternaria* spp., acetic acid, grapefruit extract, essential oils, fir oil, thyme oil

INTRODUCTION

Seed associated species of genus *Alternaria* had been shown to be a major source of severe diseases of carrot (*Daucus carota* L.). The most important among them are *Alternaria dauci* (Kühn) Groves et Skolko, causing leaf blight, and *A. radicina* Meier, Drechsler et Eddy, responsible for black root rot. Moreover, severe infection with these fungi results in seed decay and seedling damping-off [Tylkowska 1992]. *Alternaria alternata* (Fr.) Keissl., commonly occurring on seeds of many plants as saprotroph, is also considered by some researchers as weak pathogen of carrot, which may negatively affect seed germination [Tylkowska 1991]. Some fungicides, such as iprodione, can control *Alternaria* spp. on carrot seeds efficiently, but their use in organic farming is restricted. This, in turn, could have adverse implications for yield quality and quantity. Therefore, several alternative control strategies, such as non-chemical seed treatment, have been proposed. Natural products, such as organic acids, plant extracts and essential oils, may potentially replace synthetic fungicides. Some of them, because of antifungal, antibacterial and antioxidative properties, have been commonly used in medicine, food production and cosmetic industry.

Vinegar, containing 6% or 10% of acetic acid, is well known food preservative. Acetic acid naturally occurs in many fruits as metabolic intermediate and,

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together with lactic acid, is one of the main products of lactic acid bacteria (genera in the order *Lactobacilliales*) [Stiles 1996]. There are several reports that acetic acid may successfully control fungi associated with seeds [Sholberg and Gaunce 1996, Sholberg et al. 2006, Szopińska 2013].

Antimicrobial and antioxidative properties of grapefruit extract – a complex of hydroxybenzen diphenole, which contains numerous flavonoids and glycosides, namely naringenin rutinoside, hesperidin, kaempferol, dihydrokaempferol, quercetin, apigenin rutinoside, nobiletin and some proteins – are also well known [Angioni et al. 1998, Kędzia 2001, Orlikowski 2001, Cvetnić and Vladimir-Knežević 2004, Dorna et al. 2004, 2005]. Commercial products, obtained from seeds and pulp of grapefruit, contain usually 33% of an extract. They are used in foodstuff production, medicine and cosmetic industry, moreover, they have been also effectively applied in plant protection [Dorna et al. 2004, 2005, Pięta et al. 2004, 2005, 2007, Patkowska 2006, 2008, Szopińska et al. 2007].

Essential oils are volatile, complex compounds formed naturally by aromatic plants as secondary metabolites. Antibacterial, antifungal, antiviral, insecticidal and antioxidative properties of essential oils are strongly associated with their chemical composition. They contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic compounds and aliphatic compounds [Bakkali et al. 2008]. The main components of fir oil, obtained from needles and cones of *Abies alba* Mill., are α-pinene, β-pinene, camphene and limonene [Serban et al. 2011], whereas in the composition of thyme oil, obtained from *Thymus vulgaris* L. plants, thymol, *p*-cymene, γ-terpinene, carvacrol and β-caryophyllene prevail [Soković et al. 2009]. Our previous experiments and some other reports confirmed antimicrobial activity of fir oil [Serban et al. 2011], but antibacterial and antifungal activities of thyme oil are better known. Analysis performed by Zambonelli et al. [2004] showed that commercial essential oils of *T. vulgaris* contain usually 22–38% of thymol and 1–2% of carvacrol. However, the young (2 years old) plants of *T. vulgaris* may contain much more monoterpene phenols, i.e. 51.2% and 4% of thymol and carvacrol, respectively [Hudaib et al. 2002]. According to Zambonelli et al. [2004] fungicidal activity of thyme oil is correlated mainly with thymol content. The authors reported that thyme oils, as well as thymol itself, caused degeneration of fungal hyphae, i.e. increased vacuolization of the cytoplasm and accumulation of lipid bodies, undulations of the plasmalemma, and alterations of the mitochondria and endoplasmic reticulum. Several authors confirmed that thyme oil may completely inhibit or significantly limit growth of fungal plant pathogens [Zambonelli et al 1996, 2004, Daferera et al. 2003, Dorna et al. 2008, Barrera-Necha et al. 2009, Koch et al. 2010, Riccioni and Orzali 2011, Lopez-Reyes et al. 2016].

Continuous use of synthetic chemicals may result in contamination of the environment with residues of pesticides and the development of pathogen resistance, hence it is strictly limited in organic farming. Therefore, the aim of this experiment was to investigate the possibility to use natural products, i.e. acetic acid, grapefruit extract (plant origin preparation Biosept Active) and fir and thyme oils for carrot seed treatment.

MATERIALS AND METHODS

Two commercially produced carrot seed samples, cultivars 'Amsterdam 3' (sample I) and 'Flakkese 2' (sample II), obtained from TORSEED Seed Company in Toruń, were used in the experiment.

For seed treatment: acetic acid obtained from Sigma Aldrich Co., preparation Biosept Active (a. i. 33% grapefruit [*Citrus paradisi* Macfad] extract) produced by Cintamani Poland Majewscy and Koc Co. in Piaseczno, and fir (*Abies alba* Mill.) and thyme (*Thymus vulgaris* L.) essential oils obtained from Avinacea-OIL Co. in Wrocław were applied.

Seed treatment. Seed health, germination and vigour tests were performed for: untreated seeds (control), seeds soaked in distilled water for 30 min (water control for seeds soaked in the solutions of acetic acid and Biosept Acitve), seeds soaked for 30 min in 0.5, 1 and 2% solutions of acetic acid and

0.5 and 1% solutions of Biosept Active, and seeds treated with volatile compounds of fir and thyme essential oils. For essential oil treatment 1 g of seeds was placed in glass containers of 50 cm^3 volume and 1 cm^2 pieces of filter paper imbibed with 5 or 10 µl of fir or thyme oil were hung over the seeds surface. The seeds were treated at the amount of 10 µl of oil if oils were used individually, but in combined treatments, each of essential oils was applied at the amount of 5 µl. Next the containers were tightly closed with glass plugs and placed for 72 or 96 h at 20°C in darkness. After acetic acid treatment seeds were washed three times with distilled water, and next surface dried with a filter paper. Seeds soaked in distilled water (water control), as well as seeds treated with Biosept Active solutions, were surface dried with a filter paper directly after the treatment.

Seed germination test. Seed germination test was conducted at 20°C in darkness, on six replications of 50 seeds for each treatment. Seeds were placed in 9 cm diameter Petri dishes containing six layers of filter paper moistened with distilled water. The percentages of normal seedlings (germination at the first and final count), abnormal diseased seedlings and dead seeds were determined after seven and fourteen days of incubation according to the rules of International Seed Testing Association – ISTA [International Rules for Seed Testing 2012].

Seed vigour test. For vigour tests six replications of 50 seeds from each treatment were incubated for 14 days in 9 cm diameter Petri dishes on six layers of filter paper moistened with distilled water (50 seeds per dish), at 20°C in darkness. Germination rates, characterizing speed and uniformity of germination i.e.: T_1 – time to 1% of the percentage of germinating seeds (G_{max}), T_{50} – time to 50% of G_{max} and U_{75-25} – time between 25 and 75% of G_{max}, were determined on the basis of the number of seeds with visible radicle, counted daily.

Seed health analysis. Seed health was evaluated according to the ISTA rules [International Rules for Seed Testing 2014a, 2014b] by means of the deep-freeze blotter test. Two hundred seeds, five replications of 40 seeds, for each treatment were examined. Seeds were placed in 9 cm diameter Petri dishes on six layers of filter paper moistened with distilled water, 20 seeds per dish, and incubated 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.

Data analysis. SeedCalculator version 2.1 software [Jalink and Van der Schoor 1999] was applied to analyze vigour data. All results were compared by means of variance analysis followed by the Duncan's multiple range test.

RESULTS

Seed germination. In sample I, in most cases, the applied treatments did not affect seed germination at the first and final counts, as well as the percentages of abnormal diseased seedlings and dead seeds (tab. 1). A decrease in germination at the first count was observed only if seeds were treated with thyme oil for 72 h, whereas a reduction in germination at the final count was found after combined treatment with fir and thyme oils for 96 h. In sample II, all applied treatments, except treating seeds jointly with fir and thyme oils for 72 h, resulted in an increase in germination at the first count. An improvement of germination at the final count was observed only if seeds were soaked in 0.5 and 1% Biosept Active solutions. Other treatments did not affect this parameter. Soaking seeds in the solutions of acetic acid and Biosept Active, regardless of concentration, as well as treating seeds with fir oil for 96 h reduced the percentage of abnormal diseased seedlings in sample II. Moreover, soaking seeds in the solutions of acetic acid at the concentrations of 1 and 2%, and in the solutions of Biosept Active at the concentrations of 0.5 and 1%, as well as treating seeds with thyme oil for 72 h and with both oils together for 96 h decreased the percentage of dead seeds. Other treatments did not show significant effect on these parameters.

Table 1. The effect of acetic acid, grapefruit extract (Biosept Active) and essential oils on germination of carrot seeds

Treatment	Germination at the first count (%)		Germination at the final count (%)		Abnormal diseased seedlings (%)		Dead seeds (%)	
				Sample I				
Control*	71.3	bc**	78.3	b-d	10.7	a-c	3.0	ab
Water control	75.3	c	79.7	cd	9.7	a-c	2.7	ab
AA 0.5%	69.3	a-c	82.0	cd	9.0	a	4.3	ab
AA 1%	66.7	a-c	78.3	b-d	11.7	a-c	3.0	ab
AA 2%	67.7	a-c	82.0	cd	7.7	ab	4.7	ab
BA 0.5%	75.3	c	84.3	d	7.3	a	3.0	ab
BA 1%	69.3	a-c	79.7	cd	8.0	ab	1.7	a
F/10µl/72h	63.7	ab	76.3	a-d	12.3	a-c	2.7	ab
F/10µl/96h	66.7	a-c	70.3	a-b	16.0	bc	3.0	ab
T/10µl/72h	59.7	a	74.0	a-c	17.0	c	3.0	ab
T/10µl/96h	72.3	bc	76.3	a-d	11.0	a-c	3.0	ab
F+T/5+5µl/72h	75.3	c	79.3	cd	8.3	ab	5.7	ab
F+T/5+5µl/96h	64.0	ab	68.3	a	13.0	a-c	5.7	b
				Sample II				
Control	39.3	a	55.7	a	12.3	f	10.7	f
Water control	60.7	c	65.0	a-c	8.7	d-f	4.0	a-e
AA 0.5%	56.0	bc	64.7	a-c	2.0	ab	4.7	c-f
AA 1%	54.3	bc	64.7	a-c	5.7	be	2.7	a-d
AA 2%	56.7	bc	65.7	a-c	0.7	a	1.3	ab
BA 0.5%	58.7	c	72.3	c	3.0	a-c	1.7	ab
BA 1%	62.0	c	69.0	bc	2.3	a-c	0.7	a
F/10µl/72h	52.3	bc	63.7	a-c	7.0	d-f	7.7	d-f
F/10µl/96h	57.0	bc	64.7	a-c	4.0	b-d	6.3	c-f
T/10µl/72h	59.0	c	63.7	a-c	10.0	ef	2.3	a-c
T/10µl/96h	60.0	c	65.7	a-c	5.7	c-f	6.7	d-f
F+T/5+5µl/72h	46.0	ab	59.3	ab	9.3	ef	7.7	ef
F+T/5+5µl/96h	56.0	bc	62.7	a-c	6.3	c-f	3.7	b-e

*Untreated seeds
Water control – seeds soaked in distilled water for 30 min
AA 0.5%, AA 1%, AA 2% – seeds soaked in 0.5, 1 and 2% acetic acid solutions for 30 min
BA 0.5%, BA 1% – seeds soaked in 0.5 and 1% solutions of Biosept Active for 30 min
F/10µl/72h, F/10µl/96h – seeds treated with 10 µl of fir oil for 72 and 96 h
T/10µl/72h, T/10µl/96h – seeds treated with 10 µl of thyme oil for 72 and 96 h
F+T/5+5µl/72h, F+T/5+5µl/96h – seeds treated with fir and thyme oils, 5 µl of each, for 72 and 96 h
**Means in columns, for each sample separately, 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 acetic acid, grapefruit extract (Biosept Active) and essential oils on speed and uniformity of carrot seed germination

Treatment	T_1* (days)	T_{50} (days)	U_{75-25} (days)
	Sample I		
Control	1.81 ab	2.75 a-c	0.75 a
Water control	1.89 ab	2.67 a	0.70 a
AA 0.5%	1.83 ab	2.83 b-d	0.75 a
AA 1%	1.92 bc	2.81 a-c	0.74 a
AA 2%	2.18 c	2.97 d	0.71 a
BA 0.5%	2.18 c	2.90 cd	0.65 a
BA 1%	1.97 bc	2.85 b-d	0.71 a
F/10µl/72h	1.87 ab	2.80 a-c	0.72 a
F/10µl/96h	1.73 ab	2.71 ab	0.84 a
T/10µl/72h	1.94 bc	2.86 b-d	0.73 a
T/10µl/96h	1.99 bc	2.90 cd	0.75 a
F+T/5+5µl/72h	1.64 a	2.78 a-c	0.81 a
F+T/5+5µl/96h	1.87 ab	2.79 a-c	0.77 a
	Sample II		
Control	1.99 ab	2.95 a-c	0.78 a
Water control	1.91 ab	2.81 a	0.85 a
AA 0.5%	2.01 ab	2.98 a-c	0.98 a
AA 1%	2.17 b	3.20 d	1.01 a
AA 2%	2.71 c	4.27 e	1.70 b
BA 0.5%	2.09 ab	3.03 b-d	0.88 a
BA 1%	2.06 ab	3.05 b-d	0.89 a
F/10µl/72h	1.86 ab	2.96 a-c	1.01 a
F/10µl/96h	1.81 a	2.88 ab	0.87 a
T/10µl/72h	2.03 ab	2.90 a-c	0.86 a
T/10µl/96h	2.00 ab	3.09 cd	0.97 a
F+T/5+5µl/72h	1.99 ab	2.98 a-c	0.98 a
F+T/5+5µl/96h	1.81 a	2.93 a-c	0.92 a

* Time to 1% of G_{max} (percentage of germinating seeds)
T_{50} – time to 50% of G_{max}
U_{75-25} – time between 25 and 75% of G_{max}
For further explanations see table 1

Seed vigour. Acetic acid applied at the concentration of 2% negatively affected the speed of seed germination, expressed by T_1 and T_{50} parameters, in both samples (tab. 2). Furthermore, soaking seeds in 0.5% solution of Biosept Active prolonged time to 1% of G_{max} in sample I and treating seeds with 1.0% acetic acid solution increased significantly T_{50} parameter in sample II. Deterioration of the uniformity of germination was observed only in sample II, if seeds were treated with acetic acid solution at the highest concentration. Other treatments did not affect vigour of the seeds in both samples.

Seed health. Seeds of sample I were frequently infested with: *Alternaria alternata* (Fr.) Keissl., *A. radicina* Meier, Drechsler & E.D. Eddy, *Epicoccum nigrum* Link., *Fusarium* spp. and *Melanospora simplex* (Corda) D. Hawksw. (tabs 3 and 4). Other fungi, i.e.: *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko, *Bipolaris sorokiniana* Shoemaker, *Cladosporium* spp., *Penicillium* spp., *Rhizopus stolonifer* (Ehrenb.) Vuill., *Stemphylium botryosum* Wallr., *Ulocladium* spp. and *Verticillium* spp. were detected on the seeds sporadically (data shown only for selected fungi). Soaking seeds in the solutions of acetic acid increased the percentage of seeds free of fungi in relation to untreated seeds and seeds soaked in distilled water. The largest number of these seeds was observed when acetic acid at the highest concentration was applied (tab. 3). It was connected mostly with a reduction in seed infestation with *A. alternata*. The percentage of seeds infested with this fungus decreased also if seeds were soaked in 1% Biosept Active solution. None of the treatments controlled *A. dauci* on the seeds. Moreover, treating seeds with fir oil for 72 h and jointly with fir and thyme oils for 72 h increased seed infestation with this pathogen. All oil treatments increased also the percentage of seeds infected by *A. radicina*. However, soaking seeds in acetic acid solutions, regardless of concentration, resulted in a reduction in seed infestation with this fungus. In case of *Cladosporium* spp., soaking seeds in 1 and 2% acetic acid solutions decreased the percentage of seeds infested with these fungi, whereas application of 0.5% solution resulted in an increase in seed infestation. Treating seeds with fir and thyme oils individually for 72 and 96 h also favoured growth of *Cladosporium* spp. on the seeds. Soaking seeds in the solutions of acetic acid and Biosept Active reduced seed infestation with *E. nigrum*. Only acetic acid treatments controlled growth of *Fusarium* spp. on the seeds. Treating seeds with acetic acid, Biosept Active, thyme oil for 72 and 96 h, and jointly fir and thyme oils for 72 and 96 h, as well as soaking seeds in water, limited significantly seed infestation with *M. simplex*.

Seeds of sample II were mostly infested with *A. alternata* and *A. radicina* (tab. 3). Other fungi, i.e.: *A. dauci*, *B. sorokiniana*, *Cladosporium* spp., *E. nigrum*, *Fusarium* spp., *R. stolonifer*, *S. botryosum* and *Ulocladium* spp. were found on seeds sporadically and in some cases only after the treatment (tab. 4, data shown only for selected fungi). All treatments, including soaking seeds in water, resulted in an increase in the percentage of seeds free of fungi. The largest numbers of these seeds were noted after soaking seeds in the solutions of acetic acid at concentrations of 1.0 and 2.0% and in the solutions of Biosept Active at concentrations of 0.5 and 1% (tab. 3). All applied treatments decreased the incidence of *A. alternata* compared with untreated seeds and water control. Treating seeds with acetic acid and grapefruit extract reduced seed infestation with this fungus to the largest extent. The highest seed infestation with *A. dauci* was observed after soaking seeds in distilled water. This pathogen was detected also after treating seeds with thyme oil for 96 h and jointly with fir and thyme oils for 72 and 96 h, but the percentage of infected seeds was only 0.5. Soaking seeds in the solutions of acetic acid and Biosept Active, regardless of concentration, significantly decreased the percentage of seeds infected by *A. radicina* compared with untreated seeds and water control. Moreover, 0.5 and 2% acetic acid eradicated this pathogen from seeds. Soaking seeds in the solutions of Biosept Active and 2% acetic acid completely eliminated *Cladosporium* spp. from the seeds (tab. 4). However, treating seeds with 0.5% acetic acid increased significantly the incidence of these fungi in relation to both controls. Soaking seeds in distilled water resulted in an increase in seed infestation with *E. nigrum*,

Table 3. The effect of acetic acid, grapefruit extract (Biosept Active) and essential oils on the percentage of seeds free of fungi and carrot seed infestation with *Alternaria* spp. (the percentage of infested seeds)

Treatment	Seeds free of fungi		*Alternaria alternata*		*Alternaria dauci*		*Alternaria radicina*	
				Sample I				
Control	0	a	99.5	ef	1.5	a-c	16.5	b
Water control	7.5	c	89.5	c	4.5	b-e	18.0	b
AA 0.5%	15.0	d	72.0	b	2.5	a-d	3.0	a
AA 1%	21.0	d	76.0	b	0.5	ab	2.0	a
AA 2%	32.0	e	58.0	a	2.0	a-d	2.5	a
BA 0.5%	1.5	ab	96.5	de	0	a	8.5	b
BA 1%	2.5	bc	94.5	cd	2.5	a-d	7.5	b
F/10µl/72h	0	a	95.5	ef	9.0	e	37.5	c
F/10µl/96h	0	a	95.5	ef	4.5	c-e	37.5	c
T/10µl/72h	0	a	95.5	ef	3.0	b-e	40.5	c
T/10µl/96h	0	a	95.5	ef	4.0	c-e	40.0	c
F+T/5+5µl/72h	0	a	100.0	f	8.5	de	31.5	c
F+T/5+5µl/96h	0	a	95.5	ef	2.5	a-d	32.5	c
				Sample II				
Control	0	a	87.0	f	0	a	9.0	b
Water control	11.5	b	86.0	f	4.5	b	12.5	b
AA 0.5%	65.5	f	7.0	b	0	a	0	a
AA 1%	92.5	gh	3.5	ab	0	a	0.5	a
AA 2%	96.0	h	2.0	a	0	a	0	a
BA 0.5%	86.0	g	9.5	b	0	a	0.5	a
BA 1%	91.5	gh	7.5	b	0	a	0.5	a
F/10µl/72h	21.5	cd	71.0	e	0	a	7.0	b
F/10µl/96h	16.5	c	71.5	e	0	a	9.5	b
T/10µl/72h	48.0	e	38.5	c	0	a	4.5	b
T/10µl/96h	46.5	e	46.0	cd	0.5	a	8.0	b
F+T/5+5µl/72h	29.5	d	63.0	e	0.5	a	7.0	b
F+T/5+5µl/96h	32.0	d	59.0	de	0.5	a	5.5	b

For explanations see table 1

Table 4. The effect of acetic acid, grapefruit extract (Biosept Active) and essential oils on carrot seed infestation with *Cladosporium* spp. *Epicoccum nigrum*, *Fusarium* spp. and *Melanospora simplex* (the percentage of infested seeds)

Treatment	*Cladosporium* spp.		*Epicoccum nigrum*		*Fusarium* spp.		*Melanospora simplex*	
				Sample I				
Control	3.5	bc	22.5	b	8.5	d-f	28.0	d
Water control	0	a	12.5	b	15.0	ef	0	a
AA 0.5%	8.5	de	0.5	a	1.0	ab	0	a
AA 1%	0	a	0	a	1.0	a-c	0	a
AA 2%	1.0	a	1.0	a	0.5	a	0	a
BA 0.5%	3.5	b-d	2.0	a	6.5	c-f	0	a
BA 1%	2.0	ab	0.5	a	3.0	a-d	0.5	a
F/10µl/72h	10.0	ef	21.5	b	9.5	d-f	29.0	d
F/10µl/96h	9.5	ef	18.0	b	9.0	d-f	30.0	d
T/10µl/72h	20.0	g	13.5	b	4.0	a-d	3.5	b
T/10µl/96h	17.5	fg	19.0	b	5.5	b-e	8.5	c
F+T/5+5µl/72h	10.0	c-e	16.5	b	14.5	f	24.0	d
F+T/5+5µl/96h	3.5	b-d	15.0	b	4.5	b-e	14.5	c
				Sample II				
Control	3.0	bc	3.5	bc	1.0	a	0	a
Water control	5.5	c	13.5	d	12.5	b	9.5	b
AA 0.5%	22.5	d	0.5	ab	0.5	a	0	a
AA 1%	0.5	ab	0.5	ab	0	a	0	a
AA 2%	0	a	0.5	ab	0	a	0	a
BA 0.5%	0	a	0	a	0	a	0	a
BA 1%	0	a	0	a	0	a	0	a
F/10µl/72h	4.5	bc	2.5	a-c	0	a	0	a
F/10µl/96h	1.5	a-c	4.5	cd	0	a	0.5	a
T/10µl/72h	2.0	a-c	1.5	a-c	0.5	a	0	a
T/10µl/96h	2.0	a-c	3.5	b-d	0	a	7.5	b
F+T/5+5µl/72h	2.0	a-c	4.5	cd	0.5	a	0	a
F+T/5+5µl/96h	0.5	ab	3.5	bc	0	a	0	a

For explanations see table 1

Fusarium spp. and *M. simplex*. Additionally, *M. simplex* was detected on seeds treated individually with fir and thyme oils for 96 h. Soaking seeds in 0.5 and 1% Biosept Active solutions decreased seed infestation with *E. nigrum* in relation to untreated seeds and water control. Acetic acid and Biosept Active treatments effectively controlled *Fusarium* spp.

DISCUSSION

From among applied treatments acetic acid most effectively reduced carrot seed infestation with pathogenic and saprotrophic fungi, especially *A. alternata*, *A. radicina* and *Fusarium* spp., and significantly increased the percentage of seeds free of fungi. Strong antimicrobial properties of acetic acid, mostly applied in gaseous form, confirmed many authors. There are reports that vapour of acetic acid efficiently controlled common bunt (*Tilletia* spp.) in wheat [Sholberg et al. 2006], decay of tomato fruits caused by *Alternaria alternata* Fr. Keisl. and *Botrytis cinerea* Pers. [Alawlaqi and Alharbi Asmaa 2014] and a growth of *Aspergillus flavus* Link ex Fries during canola, corn, rice, and wheat seed storage [Sholberg and Gaunce 1996]. Szopińska [2013] found that soaking common zinnia (*Zinnia elegans* Jacq.) seeds in 1, 2.5 and 5% acetic acid solutions significantly reduced their infestation with *A. alternata*, *Alternaria zinniae* M.B. Ellis and *Fusarium* spp., but negatively affected seed germination and vigour, especially if 2.5 and 5% solutions were applied. Phytotoxic effect of acetic acid observed also Pasini et al. [1997] on roses sprayed in the field with 0.25% and 0.5% acetic acid against powdery mildew (*Podosphaera pannosa* (Wallr.) de Bary). Success of acetic acid treatment seems to be strongly associated with a form of its application (gaseous or liquid), its concentration in the solution or in the air, and morphological characteristic of treated seeds. Generally, the pathogens which contaminate seed/fruits surface or infect external seed/fruits tissues (seed coat and pericarp), such as fungi of genus *Alternaria*, are more susceptible for this type of treatment. Dipper penetration of acid into seed tissues may result in a damage of the embryo. Moreover, we observed that the fruits, such as schizocarps of carrot or achenes of zinnia, better than true seeds tolerate acetic acid treatment. Presumably the pericarp additionally protects the embryo from toxicity of the acid. Despite this, our previous experiments showed that the seeds/fruits have to be washed after the treatment, because prolonged contact with acetic acid negatively affected their viability.

Grapefruit extract, unlike acetic acid, effectively controlled fungi associated with carrot seeds mostly in one sample, nevertheless in this case an enhancement of seed health was connected with a significant improvement in final germination. Mazur and Nawrocki [2007] reported that spraying carrot plants three times during vegetation with grapefruit extract (Biosept 33 SL at the concentration of 0.2%) effectively controlled Alternaria blight in the field. Moreover, in the laboratory conditions Biosept 33 SL significantly limited the size of necrosis of petioles infected by *A. alternata* and *A. radicina.* Grapefruit extract was also effectively applied against seed-borne *Botrytis* spp. on onion [Dorna et al. 2004, 2005], to protect leguminous crops against *Pythium oligandrum* [Pięta et al. 2004, 2005, 2007, Patkowska 2006, Patkowska 2008] and to control *Alternaria brassicicola*, *Botrytis aclada* and *B. cinerea*, and *A. zinniae* on cabbage, onion and zinnia seeds, respectively [Szopińska et al. 2007]. Grapefruit seed extract include many antimicrobial and antioxidative compounds [Angioni et al. 1998, Kędzia 2001, Orlikowski 2001, Cvetnić and Vladimir-Knežević 2004] and probably not only a reduction in seed infestation with fungi but also an activity of antioxidants was responsible for the improvement in carrot seed germination.

There are several reports about antimicrobial properties of essential oils. Especially antibacterial and antifungal activities of thyme oil components are well known [Soković et al. 2009]. Thyme oil completely inhibited or significantly controlled the growth of fungal plant pathogens, such as: *Botrytis cinerea* Pers., *Colletotrichum lindemuthianum* (Sacc. et Magn.) Briosi et Cav., *Fusarium culmorum* (Wm. G. Sm.) Sacc., *F. solani* (Mart.) Sacc, *F. oxysporum* Schltdl., *Gibberella zeae* (Schwein.) Petch

(syn. *F. graminearum* Schwabe), *Globisporangium ultimum* (Trow) Uzuhashi, Tojo et Kakish. (syn. *Pythium ultimum* Trow.), *Mycosphaerella rabiei* Kovatsch. ex Gruyter (syn. *Ascochyta rabiei* (Pass.) Labr, *Pyrenophora chaetomioides* Speg. (syn. *Drechslera avenae* (Eidam) Scharif), and *Rhizoctonia solani* Kühn. [Zambonelli et al. 1996, 2004, Daferera et al. 2003, Barrera-Necha et al. 2009, Riccioni and Orzali 2011]. Some experiments revealed that thyme oil may be effectively used also to control *Alternaria* spp. on carrot seeds [Dorna et al. 2008, Koch et al. 2010, Riccioni and Orzali 2011, Lopez-Reyes et al. 2016]. Properties of fir oil are less known, however its chemical composition may indicate antimicrobial activity. *In vitro* experiment of Serban et al. [2011] showed inhibitory activity of fir oil against *Candida albicans* strain ATCC 10231. Unfortunately, in our experiment, essential oils treatments resulted in a significant increase in seed infestation with *A. radicina*, *Cladosporium* spp. and *M. simplex* in sample I, although generally they did not affect negatively seed germination. On the other hand, in sample II in most cases a decrease in the incidence of *A. alternata*, an increase in the percentage of seeds free of fungi and an improvement of germination at the first count was observed after essential oils treatments. This discrepancies between results obtained for examined samples point out a necessity of further research.

CONCLUSIONS

1. Acetic acid treatment significantly increased the percentage of seeds free of fungi and decreased the incidence of *A. alternata* and *A. radicina* in both samples, and *E. nigrum* and *Fusarium* spp. in sample I. Treating seeds with acetic acid solutions did not affect adversely seed germination, however, at the highest concentration it deteriorated seed vigour in both samples.

2. Biosept Active applied at the concentration of 1% decreased the percentage of seeds infested with *A. alternata* and *E. nigrum* in sample I, and at the concentrations of 0.5 and 1% reduced seed infestation with *A. alternata*, *A. radicina* and *E. nigrum* in sample II. Moreover, especially in sample II, Biosept Active treatments improved seed germination, without negative effects on seed vigour.

3. Generally, treating seeds with essential oils improved germination at the first count, increased the percentage of seeds free of fungi and decreased the incidence of *A. alternata* in sample II, however in sample I these treatments favoured growth of *A. radicina*, and, in some cases, *A. dauci*.

REFERENCES

Alawlaqi, M.M., Alharbi Asmaa, A. (2014). Impact of acetic acid on controlling tomato fruit decay. Life Sci. J., 11(3s), 114–119.

Angioni, A., Cabras, P., Hallewin, D., Pirisi, F.M., Reniero, F., Schirra, M. (1998). Synthesis and inhibitory activity of 7-geranoxycoumarin against *Penicillium* species in *Citrus* fruit. Phytochemistry, 47(8), 1521–1525.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008). Biological effects of essential oils – a review. Food Chem. Toxicol., 46, 446–475. DOI: 10.1016/j.fct.2007.09.106.

Barrera-Necha, L.L., Garduño-Pizaña, C., Garcia-Barrera, L.J. (2009). *In vitro* antifungal activity of essential oils and their compounds on mycelial growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder and Hanson. Plant Pathol. J., 8(1), 17–21.

Cvetnić, Z., Vladimir-Knežević, S. (2004). Antimicrobial activity of grapefruit seed and pulp ethanolic extract. Acta Pharm., 54, 243–250.

Daferera, D.J., Ziogas, B.N., Polissiou, M.G. (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Prot., 22, 39–44. DOI: 10.1016/S0261-2194(02)00095-9.

Dorna, H., Górski, R., Szopińska, D., Tylkowska, K. (2008). Zdrowotność nasion marchwi traktowanych naturalnymi olejkami eterycznymi [Health of carrot seeds treated with natural essential oils]. Prog. Plant Prot., 48(2), 710–714.

Dorna, H., Tylkowska, K., Shan Wenyan, Szopińska, D. (2004). Wpływ preparatów pochodzenia roślinnego na zdrowotność, kiełkowanie i wigor nasion oraz wschody

cebuli (*Allium cepa* L.). Folia Univ. Agric. Stetin. 239, Agricultura, 95, 69–74.

Dorna, H., Tylkowska, K., Wei Yahong, Marcinek, R. (2005). Germination and health of onion (*Allium cepa*) seeds after priming combined with chemical or biological treatments. Phytopathol. Pol., 37, 69–81.

Hudaib, M., Speroni, E., Di Pietra, A.M., Cavrini, V. (2002). GC/MS evaluation of thyme (*Thymus* v*ulgaris* L.) oil composition and variations during the vegetative cycle. J. Pharm. Biomed. Anal., 29, 691–700.

International Rules for Seed Testing (2012). International Seed Testing Association (ISTA), Bassersdorf, Switzerland.

International Rules for Seed Testing (2014a). Annexe to Chapter 7. Seed Health Testing Methods 7-001a: Blotter method for the detection of *Alternaria dauci* on *Daucus carota* (carrot). International Seed Testing Association (ISTA), Bassersdorf, Switzerland.

International Rules for Seed Testing (2014b). Annexe to Chapter 7. Seed Health Testing Methods 7-002a: Blotter method for the detection of *Alternaria radicina* on *Daucus carota* (carrot). ISTA, Bassersdorf, Switzerland.

Jalink, H., Van der Schoor, R. (1999). Seed Calculator 2.1. License number: 100200122. Plant Research International, Wageningen, the Netherlands.

Kędzia, I. (2001). Działanie Citroseptu na bakterie beztlenowe występujące w zakażeniach dróg oddechowych. Wiad. Ziel., 3, 20–21.

Koch, E., Schmitt, A., Stephan, D., Kromphardt, C., Jahn, M., Krauthausen, H.J., Forsberg, G., Werner, S., Amein, T., Wright, S.A.I., Tinivella, F., Gullino, M.L., Roberts, S.J., van der Wolf, J., Groot, S.P.C. (2010). Evaluation of non-chemical seed treatment methods for the control of *Alternaria dauci* and *A. radicina* on carrot seeds. Eur. J. Plant Pathol., 127, 99–112. DOI: 10.1007/s10658-009-9575-3.

Lopez-Reyes, J.G., Gilardi, G., Garibaldi, A., Gullino, M.L. (2016). In vivo evaluation of essential oils and biocontrol agents combined with hot water treatments on carrot seeds against *Alternaria radicina.* J. Phytopathol., 164, 131–135. DOI: 10.1111/jph.12400.

Machado, J.C., Langerak, C.J., Jaccoud-Filho, D.S. (2002). Seed-borne Fungi: A contribution to routine seed health analysis. ISTA, Bassersdorf, Switzerland.

Mathur, S.B., Kongsdal, O. (2003). Common laboratory seed health testing methods for detecting fungi. ISTA,, Bassersdorf, Switzerland.

Mazur, S., Nawrocki, J. (2007). Wykorzystanie związków naturalnych w ochronie marchwi przed Alternariozą [The application of natural compounds in protection of carrot against Alternaria blight]. Rocz. AR Pozn. 383, Ogrodnictwo, 41, 565–569.

Orlikowski, L.B. (2001). Effect of grapefruit extract on development of *Phytophthora cryptogea* and control of foot rot of gerbera. J. Plant Prot. Res., 41(3), 288–294.

Pasini, C., D'Aquila, F., Curir, P., Gullino, M.L. (1997). Effectiveness of antifungal compounds against rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) in glasshouses. Crop Prot., 16(3), 251–256.

Patkowska, E. (2006). Effectiveness of grapefruit extract and *Pythium oligandrum* in the control of bean and peas pathogens. J. Plant Prot. Res., 46(1), 15–28.

Patkowska, E. (2008). The application of chitosan, *Pythium oligandrum* and grapefruit extract in the protection of common bean (*Phaseolus vulgaris* L.) from soil-borne phytopathogens. Prog. Chem. Appl. Chitin Deriv. 13, 133–140.

Pięta, D., Pastucha, A., Patkowska, E. (2007). A possibility of using grapefruit extract, chitosan and *Pythium oligandrum* to protect soybean (*Glycine max* (L.) Merrill) from pathogens. Prog. Chem. Appl. Chitin Deriv., 12, 197–203.

Pięta, D., Patkowska, E., Pastucha, A. (2004). Oddziaływanie biopreparatów na wzrost i rozwój niektórych grzybów chorobotwórczych dla roślin motylkowatych. Acta Sci. Pol. Hortorum Cultus, 3(2), 171–177.

Pięta, D., Patkowska, E., Pastucha, A. (2005). The protective effect of biopreparations applied as the dressing for common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.). Acta Sci. Pol. Hortorum Cultus, 4(2), 59–67.

Riccioni, L., Orzali, L. (2011). Activity of tea tree (*Melaleuca alternifolia,* Cheel) and thyme (*Thymus vulgaris,* Linnaeus.) essential oils against some pathogenic seed borne fungi. J. Essent. Oil Res., 23, 43–47. DOI: 10.1080/10412905.2011.9712280.

Serban, E.S., Ionescu, M., Matinca, D., Maier, C.S., Bojita, M.T. (2011). Screening of the antibacterial and antifungal activity of eight volatile essential oils. Farmacia, 59(3), 440–446.

Sholberg, P.L., Gaudet, D.A., Puchalski, B., Randall, P. (2006). Control of common bunt (*Tilletia tritici* and *T. laevis*) of wheat (*Triticum aestivum* cv. 'Laura') by

fumigation with acetic acid vapour. Can. J. Plant. Sci., 86, 839–843. DOI: 10.4141/P05-176.

Sholberg, P.L., Gaunce, A.P. (1996). Fumigation of high moisture seed with acetic acid to control storage mold. Can. J. Plant Sci., 76, 551–555. DOI: 10.4141/cjps96-100.

Soković, M.D., Vukojević, J., Marin, P.D., Brkić, D.D., Vajs, V., van Griensven, L.J.L.D. (2009). Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. Molecules, 14, 238–249. DOI: 10.3390/molecules14010238.

Stiles, M.E. (1996). Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek, 70(2–4), 331–345.

Szopińska, D. (2013). The effects of organic acids treatment on germination, vigour and health of zinnia (*Zinnia elegans* Jacq.) seeds. Acta Sci. Pol., Hortorum Cultus, 12(5), 17–29.

Szopińska, D., Dorna, H., Tylkowska, K. (2007). The effects of grapefruit extract on germination, vigour and health of cabbage, onion and zinnia seeds. Rocz. AR Pozn., 383, Ogrodnictwo, 41, 631–636.

Tylkowska, K. (1991). The influence of *Alternaria alternata* (Fr.) Keissler on carrot (*Daucus carota* L.) seed germination. Phytopathol. Pol., 1(13), 14–18.

Tylkowska, K. (1992). Carrot seed-borne diseases caused by *Alternaria* species. In: *Alternaria*, biology, plant diseases and metabolites, Chełkowski, J., Visconti, A. (eds). Elsevier Science Publishers B.V., Amsterdam, the Netherlands, 337–352.

Zambonelli, A., Zechini D'Aulerio, A., Severi, A., Benvenuti, S., Maggi, L., Bianchi, A. (2004). Chemical composition and fungicidal activity of commercial essential oils of *Thymus vulgaris* L. J. Ess. Oils Res., 16, 69–74. DOI: 10.1080/10412905.2004.9698653.

Zambonelli, A., Zechini D'Aulerio, A., Bianchi, A., Albasini, A. (1996). Effects of essential oils on phytopathogenic fungi *in vitro*. J. Phytopathology, 144, 491–494. DOI: 10.1111/j.1439-0434.1996.tb00330.x.