Romuald GÓRSKI¹, Dorota SZOPIŃSKA^{2*}, Hanna DORNA², Agnieszka ROSIŃSKA²
Zuzanna STEFAŃSKA¹ and Jolanta LISIECKA³

EFFECTS OF PLANT EXTRACTS AND DISINFECTANT HUVA-SAN TR 50 ON THE QUALITY OF CARROT (*Daucus carota* L.) SEEDS

Abstract: The aim of the study was to determine the effect of *Aloe vera* (L.) Burm.f. (*Aloe*) and *Morinda citrifolia* L. (Indian mulberry) plant extracts and disinfectant Huva-San TR 50 (hydrogen peroxide stabilized with silver ions) on carrot seed quality. The seeds of two carrot cultivars Amsterdam (sample I) and Berlikumer 2 (sample II), were soaked in solutions of *Aloe* and Indian mulberry extracts and Huva-San TR 50 at concentrations of 0.025 %, 0.05 % and 0.1 % for 30 min. Untreated seeds and seeds soaked in distilled water for 30 min were used as controls. Seed germination was assessed after 7 and 14 days of incubation. The speed and uniformity of seed germination (vigour) were evaluated. Mycological analysis was performed using a deep-freeze blotter test. Sample I was characterized by lower germination at the first and the final counts than sample II and higher seed infestation with *Alternaria radicina*. Treating seeds of sample I with *Morinda citrifolia* plant extract at concentrations of 0.025 and 0.05 % reduced their infestation with *Alternaria alternata* and *A. radicina*, increased germination at the first and the final counts, and did not affect seed vigour. The effects of *Aloe* extract and disinfectant Huva-San TR 50 on seed quality parameters varied depending on their concentration. Hence, further studies are necessary to establish the optimal conditions for carrot seed treatment.

Keywords: *Aloe vera*, *Morinda citrifolia*, hydrogen peroxide, seed germination and vigour, fungi

Introduction

The most severe fungal diseases of carrot (*Daucus carota* L.) are black rot of roots and leaf blight caused by seed-borne *Alternaria radicina* Meier, Drechsler et Eddy and *Alternaria dauci* (Kühn) Groves et Skolko, respectively. Moreover, carrot seeds are commonly and abundantly infested with *Alternaria alternata* (Fr.) Keissler, which may be potentially pathogenic to this plant [1]. Fungi from genus *Alternaria* are usually a reason of low carrot seed germination and poor seedling establishment [2]. Synthetic fungicides are commonly used to control seed-transmitted pathogens, but global need for more organic production, including discarding standard pesticides, has resulted in the search for novel, alternative methods of seed treatment.

Aloe vera (L.) Burm.f. (*Aloe*) and *Morinda citrifolia* L. (Indian mulberry), have been used traditionally in an alternative medicine due to proven antihelminthic, analgesic,

¹ Department of Entomology and Environmental Protection, Poznań University of Life Sciences, ul. J.H. Dąbrowskiego 159, 60-594 Poznań, Poland, +48 618 466 336, email: romuald.gorski@up.poznan.pl

² Department of Phytopathology, Seed Science and Technology, Poznań University of Life Sciences, ul. J.H. Dąbrowskiego 159, 60-594 Poznań, Poland, +48 618 466 382

³ Department of Vegetable Crops, Poznań University of Life Sciences, ul. J.H. Dąbrowskiego 159, 60-594 Poznań, Poland, email: jolanta.lisiecka@up.poznan.pl

*Corresponding author: dorota.szopinska@up.poznan.pl

antimicrobial, anti-tumour, anti-inflammatory and immunostimulant properties and potent antioxidant activity [3, 4].

Two components are obtained from fresh *A. vera* leaves: a bitter, yellow liquid fraction and a mucilaginous pulp from the parenchymatous tissue, commercially designated as a gel [5]. Both, leaf pulp and exudate, contain over 75 active ingredients, among others anthraquinones (aloe-emodin, aloetic-acid, anthranol, aloin A and B, isobarbaloin, emodin, and ester of cinnamic acid), known for their anti-microbial properties, as well as phenolic compounds and some enzymes, like glutathione peroxidase and superoxide dismutase, which may be responsible for anti-oxidant effect of this plant [6]. Antifungal properties of *Aloe* against seed-associated fungi has been frequently confirmed. It has been found that extracts of this plant may control growth of: *Alternaria alternata*, *A. citri*, *A. tenuissima*, *Aspergillus clavatus*, *A. flavus*, *A. niger*, *Botrytis cinerea*, *B. gladiolorum*, *Cladosporium* spp., *Colletotrichum coccodes*, *C. gleosporioides*, *Drechslera havaiensis*, *Fusarium oxysporum*, *F. proliferatum*, *F. subglutinans*, *F. verticillioides*, *Heterosporium pruneti*, *Penicillium chrysogenum*, *P. digitatum*, *P. expansum*, *P. funiculosum*, *P. gladioli*, *P. oxalicum*, *Rhizoctonia solani* and *Rhizopus stolonifer* [7-15]. Reports about activity of *Aloe* extracts against field fungi are less numerous than those related to storage fungi, and *in vitro* assays prevail over *in vivo* experiments.

Morinda citrifolia fruit extract contains many antibacterial and antifungal compounds, such as: scopoletin, anthraquinones, terpenes and polysaccharides (glucuronic acid, galactose, arabinose, rhamose, glycosides, trisaccharide fatty acid ester) [4]. The reports about antifungal properties of this extract are related mostly to human pathogens, such as: *Aspergillus niger*, *A. fumigatus* and *Candida albicans* [16-19]. Nevertheless, there are also some information about activity of Indian mulberry extract against plant associated fungi and bacteria. Under *in vitro* conditions the extract has been found antifungal against *Alternaria alternata*, *Aspergillus flavus* and *A. niger*, isolated from groundnut (*Arachis hypogaea* L.) fruits [20]. *Morinda* methanol extract inhibited the growth of *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp. colonies by 50 % [17]. *In vitro* assay revealed antibacterial activity of Indian mulberry fruit and leaves extracts against two different isolates of *Ralstonia solanacearum*, the pathogenic bacterium causing bacterial wilt of solanaceous plants [21]. Moreover, *Morinda* leaf extract inhibited the growth of *Colletotrichum acutatum* colony on PDA medium [22].

Huva-San TR 50 is a disinfectant based on hydrogen peroxide (H_2O_2), which is stabilized with silver ions. Substantial antibacterial and antifungal properties of H_2O_2 resulted from high oxidative reactivity of this compound [23-25]. It is traditionally used in medicine and food industry as disinfectant, however, there are many reports, that treating seeds with H_2O_2 may also effectively control seed-associated fungi, such as: *A. alternata*, *A. dauci* and *A. radicina* [26], *Alternaria zinniae* [27], *Sclerospora graminicola* [28], *Fusarium* spp. [27], *Peronospora tabacina* [29], *Tilletia controversa* and *T. tritici* [30]. Additionally, the results of numerous experiments revealed that H_2O_2 treatment may improve seed germination [31]. This beneficial effect has been observed for: barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) [30], carrot [26], eastern gamagrass (*Tripsacum dactyloides* L.) [32], eggplant (*Solanum melongena* L.) [33], maize (*Zea mays* L.) [34], muscadine (*Vitis rotundifolia* Michx.) [35], pea (*Pisum sativum* L.) [36], rice (*Oryza sativa* L.) [37], watermelon (*Citrullus lanatus* (Thunb.) Mansf.) [38], and zinnia (*Zinnia elegans* Jacq.) [39]. Although, silver plays a role of H_2O_2 stabilizer in Huva-San TR 50 preparation, it is also itself characterized by strong antimicrobial

properties, known from ancient times. For years the most common form of silver application was silver nitrate (AgNO_3), however nowadays, silver nanoparticles are receiving increasing attention in the field of agriculture [40-42]. It has been proved that they may effectively inhibit growth of several fungi, such as: *Aspergillus* spp., *Penicillium* sp. and *Rhizopus* sp., but, with a growing concentration they may also negatively affect seed germination [42-44].

The effectiveness of pure H_2O_2 against pathogenic *Alternaria* spp. associated with carrot seed, as well as its beneficial influence on seed germination, have been proven before, however, there is no information how H_2O_2 stabilized with silver ions may affect carrot seed quality. Moreover, despite of numerous reports about antifungal properties of *Aloe* and Indian mulberry extracts, their ability to control seed-borne carrot pathogens has not been tested yet. Therefore, the aim of this experiment was to determine the effect of disinfectant Huva-San TR 50, as well as *A. vera* and *M. citrifolia* plant extracts on germination, vigour and health of carrot seeds.

Material and methods

Material

Two standard samples of carrot seeds, cv. Amsterdam (sample I) and cv. Berlikumer 2 (sample II), obtained from Torseed S.A., were used in the experiment. *Aloe vera* (L.) Burm.f. leaf extract (*A. vera* leaf juice - 99.5 %, citric acid - 0.3 %, sodium benzoate - 0.1 %, potassium sorbate - 0.05 %), *Morinda citrifolia* L. fruit extract (*M. citrifolia* fruit extract - 1.75-3 %, propylene glycol - 70-90 %, aqua - 10-30 %, sodium benzoate 0.1-0.3 %, potassium sorbate - 0.1-0.3 %), and disinfectant Huva-San TR 50 (hydrogen peroxide - 50 %, stabilized with silver ions - 0.036 %) were used for seed treatment.

Methods

The untreated seeds were soaked in Huva-San TR 50 and plant extracts solutions (1 g of seeds per 100 ml of the solution) for 30 min. Huva-San TR 50 was applied at concentrations of 0.025, 0.05 and 0.1 %, recommended by the producer. The same concentrations were used for plant extracts. Controls included untreated seeds (control) and seeds soaked in distilled water for 30 min (water control). Seeds treated with Huva-San TR 50 and plant extracts, as well as seeds soaked in water, after treatment were drained on the sieve and next surface dried for 30 min on sterilized blotter paper. Seed germination, vigour and health were evaluated for untreated and treated seeds.

Seed germination was evaluated according to ISTA Rules [45]. For each treatment 300 seeds (six replicates of 50 seeds) were tested. Seeds were placed in 9 cm diameter Petri dishes (50 seeds per dish), on 6 layers of blotter paper moistened with distilled water, and incubated at 20 °C in darkness for 14 days. Germination at the first count (the percentage of normal seedlings) was evaluated after 7 days of incubation, while germination at the final count (total percentage of normal seedlings), the percentages of abnormal diseased seedlings, abnormal deformed seedlings, fresh seeds and dead seeds were determined after 14 days. Additionally, the percentage of germinating seeds ($G_{\max}$) was calculated on the base of seed vigour test.

To evaluate seed vigour six replicates of 50 seeds were tested for each treatment [46]. Seeds were placed in 9 cm diameter Petri dishes (50 seeds per dish), on 6 layers of blotter paper moistened with distilled water, and incubated at 20 °C in darkness. Germinating

seeds (with the radicle at least 1 mm long) were counted daily for 14 days. The speed and uniformity of germination i.e. T_{10} - time to 10 % of $G_{\max}$, T_{75} - time to 75 % of $G_{\max}$, MGT - mean germination time, U_{75-25} - time between 25 and 75 % of $G_{\max}$ were calculated using Germinator software [47].

Deep-freeze blotter test was applied to assess seed infestation with fungi. The analysis was performed on 200 seeds (four replicates of 50 seeds) for each treatment. The seeds were placed in 9 cm diameter Petri dishes on six layers of blotter moistened with distilled water, 20 seeds per dish. The seeds were incubated for three days at 20 °C in darkness, next frozen at 20 °C for 24 h, and then incubated at 20 °C under 12 h alternating cycles of NUV (Near Ultraviolet) light and darkness. Fungi were determined on the base of their growth and sporulation using a stereomicroscope and a compound microscope. *Alternaria dauci* and *A. radicina* were identified according to ISTA methodology [48, 49] and other fungi by means of ISTA manuals for identification of seed-borne pathogens and saprotrophs [50, 51]. The percentages of seeds infested with individual fungi were determined.

The results obtained were evaluated by one-way analysis of variance followed by Duncan's multiple range test, at a level $\alpha = 0.05$. Parameters characterising seed vigour were calculated using Germinator software [46].

Results

Tested samples varied significantly in seed quality. Sample I was characterized by lower germination at the first and the final counts than sample II, which was correlated with higher numbers of abnormal diseased seedlings and dead seeds (Table 1). The decrease in the percentage of germinating seeds ($G_{\max}$) in relation to untreated seeds, was observed only when sample II seeds were soaked in 0.1 % solution of *Aloe* extract. Other treatments did not affect this parameter.

Treating seeds with Indian mulberry extract, in all applied concentrations, improved seed germination at the first and the final counts in sample I, whereas did not affect these parameters in sample II. However, the highest values of both parameters were observed when 0.025 % solution of the extract was used. The significant increase in germination at the first count, as well as germination at the final count, was observed also when seeds of sample I were treated with 0.1 % *Aloe* extract. Moreover, treating seeds of sample I with *Morinda* extract solutions at all concentrations resulted in the significant decrease in the percentage of abnormal diseased seedlings, and in case of 0.025 and 0.05 % solutions, the percentage of dead seeds was reduced too. The decrease in the number of dead seeds in this sample was also observed after treatment with Huva-San TR 50 at concentrations of 0.025 and 0.1 %, and after soaking seeds in 0.1 % *Aloe* extract. The increase in the percentage of fresh seeds was statistically significant when sample I seeds were treated with 0.025 and 0.1 % solutions of Huva-San TR 50 and 0.025 % solutions of both plant extracts. In sample II the increase in the percentage of fresh seeds was found after soaking the seeds in 0.1 % solution of *Aloe* extract and 0.05 % solution of *Morinda* extract. Although none of the applied treatments affected germination at the final count of sample II seeds, the decrease in the percentage of abnormal diseased seedlings was recorded for seeds treated with 0.1 % *Aloe* and 0.025 % *Morinda* extracts.

Table 1

Effects of plant extracts and Huva-San TR 50 on seed germination

Treatments	$G_{\max}$ [%]	Germination at the first count [%]	Germination at the final count [%]	Abnormal diseased seedlings [%]	Abnormal deformed seedlings [%]	Fresh seeds [%]	Dead seeds [%]
Sample I							
C	78.7 ab	26.0 a	27.7 a	49.3 d	0.3 a	8.0 a	14.7 c
WC	77.7 ab	31.3 a-c	35.3 a-c	38.3 b-d	0.3 a	12.7 a-c	13.3 bc
A 0.025 %	81.7 b	26.0 a	28.3 ab	39.3 b-d	1.7 a	18.7 c	12.0 a-c
A 0.05 %	81.7 b	29.0 ab	31.0 ab	43.0 cd	1.3 a	14.0 a-c	10.7 a-c
A 0.1 %	79.3 b	34.6 bc	38.3 bc	40.3 c-d	0.3 a	11.3 a-c	9.7 ab
M 0.025 %	79.3 b	46.3 d	49.0 d	24.3 a	1.3 a	16.0 bc	9.3 ab
M 0.05 %	70.0 a	37.6 c	44.0 cd	32.7 a-c	1.3 a	13.7 a-c	8.3 a
M 0.1 %	74.3 ab	39.0 cd	43.0 cd	31.3 ab	1.0 a	13.0 a-c	11.7 a-c
HS 0.025 %	74.3 ab	24.3 a	34.7 a-c	38.3 b-d	1.0 a	17.3 bc	8.3 ab
HS 0.05 %	79.7 b	31.3 a-c	34.0 a-c	39.0 b-d	0.7 a	11.0 ab	15.3 c
HS 0.1 %	79.0 ab	24.0 a	36.7 a-c	37.7 b-d	0.7 a	16.7 bc	8.3 a
Sample II							
C	81.0 b	55.3 a	65.7 a	14.0 c	0 a	10.0 a	10.3 a
WC	77.0 ab	54.3 a	64.3 a	13.0 c	0 a	13.7 ab	9.0 a
A 0.025 %	77.3 ab	61.7 a	68.3 a	10.0 a-c	0 a	15.0 ab	6.7 a
A 0.05 %	76.7 ab	57.0 a	63.7 a	11.0 a-c	0 a	16.3 ab	9.0 a
A 0.1 %	74.0 a	61.3 a	68.3 a	5.7 a	0 a	18.7 b	7.3 a
M 0.025 %	79.7 ab	58.7 a	68.7 a	6.3 ab	0.3 b	16.0 ab	8.7 a
M 0.05 %	77.7 ab	56.3 a	66.0 a	8.3 a-c	0 a	18.3 b	7.3 a
M 0.1 %	79.0 ab	60.0 a	65.3 a	11.7 bc	0 a	13.3 ab	9.7 a
HS 0.025 %	76.3 ab	53.7 a	60.3 a	13.3 c	0 a	15.3 ab	11.0 a
HS 0.05 %	78.7 ab	57.7 a	66.0 a	11.0 a-c	0 a	13.0 a	10.0 a
HS 0.1 %	82.7 b	52.0 a	62.0 a	10.7 a-c	0 a	16.0 ab	11.3 a

Means in columns, for each sample separately, followed by the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's test. $G_{\max}$ - the percentage of germinating seeds, C - control - untreated seeds, WC - water control - seeds soaked in distilled water for 30 min, M 0.025 %, M 0.05 %, M 0.1 % - seeds soaked in 0.025 %, 0.05 % and 0.1 % *Morinda* extracts, respectively, A 0.025 %, A 0.05 %, A 0.1 % - seeds soaked in 0.025 %, 0.05 % and 0.1 % *Aloe* extracts, respectively, HS 0.025 %, HS 0.05 %, HS 0.1 % - seeds soaked in 0.025 %, 0.05 % and 0.1 % Huva-San TR 50 solutions, respectively

Generally, the treatments did not affect speed of seed germination in both samples (Table 2). The significant deterioration of mean germination time (*MGT*) was observed only when sample I seeds were treated with 0.1 % solution of *Aloe* extract, while soaking in 0.05 % solution of this extract significantly improved vigour of sample II seeds, expressed by T_{75} parameter. None of the treatments affected uniformity (U_{75-25}) of seed germination, as well as T_{10} parameter, in both samples.

Alternaria alternata (Fr.) Keissler was recorded on almost all untreated seeds of both samples, but significantly higher seed infestation with *Alternaria radicina* Meier, Drechsler & E.D. Edd, *Cladosporium* spp., *Melanospora simplex* (Corda) D. Hawksw. and *Stemphylium botryosum* Wallr. was observed in sample I (Table 3).

On the contrary, a higher number of seeds infested with *Fusarium* spp. was detected in sample II. *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko was observed only in sample I, and infected a relatively low percentage of seeds (maximum 1.5 %). Additionally, the pathogen was not found on untreated seeds, but in case when seeds were treated with 0.025 % and 0.05 % Huva-San TR 50 and 0.05 % and 0.1 % *Aloe* extract. Soaking sample I

seeds in the solutions of both plant extracts, regardless of their concentration, significantly limited seed infestation with *A. alternata* in relation to control. Treating seeds with 0.025 % Huva-San TR 50, 0.05 % *Aloe* extract and 0.025 % and 0.05 % Indian mulberry extract decreased significantly the percentage of seeds infected by *A. radicina* in this sample. On the other hand, none of the treatments affected seed infestation with *Alternaria* spp. in sample II. Fungi from genus *Cladosporium* were detected on 29.5 % and 4.5 % of untreated seeds of samples I and II, respectively.

Table 2

Effects of plant extracts and Huva-San TR 50 on seed vigour

Treatments	Speed of germination [days]			Uniformity of germination [days]
	T_{10}	T_{75}	MGT	U_{75-25}
Sample I				
C	2.18 ab	3.13 ab	2.97 ab	0.82 ab
WC	2.11 a	3.18 ab	2.92 ab	0.82 ab
A 0.025 %	2.17 ab	3.12 ab	2.83 a	0.73 a
A 0.05 %	2.13 a	3.12 ab	2.83 a	0.73 a
A 0.1 %	2.32 b	3.38 b	3.15 c	0.81 ab
M 0.025 %	2.09 a	3.07 a	2.84 a	0.75 ab
M 0.05 %	2.18 ab	3.19 ab	2.90 ab	0.75 ab
M 0.1 %	2.18 ab	3.29 ab	3.03 bc	0.85 ab
HS 0.025 %	2.00 a	3.25 ab	2.92 ab	0.95 b
HS 0.05 %	2.09 a	3.14 ab	2.80 a	0.75 ab
HS 0.1 %	2.05 a	3.12 ab	2.82 a	0.80 ab
Sample II				
C	1.82 a	2.91 b	2.64 a	0.84 a
WC	1.82 a	2.91 b	2.66 a	0.85 a
A 0.025 %	1.82 a	2.54 ab	2.49 a	0.58 a
A 0.05 %	1.78 a	2.58 a	2.47 a	0.64 a
A 0.1 %	1.73 a	2.65 ab	2.43 a	0.71 a
M 0.025 %	1.84 a	2.67 ab	2.53 a	0.65 a
M 0.05 %	1.89 a	2.66 ab	2.53 a	0.61 a
M 0.1 %	1.77 a	2.73 ab	2.50 a	0.74 a
HS 0.025 %	1.88 a	2.58 ab	2.58 a	0.59 a
HS 0.05 %	1.80 a	2.79 ab	2.64 a	0.79 a
HS 0.1 %	1.81 a	2.61 ab	2.49 a	0.63 a

Means in columns, for each sample separately, followed by the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's test. T_{10} - time to 10 % of G_{max} , T_{75} - time to 75 % of G_{max} , MGT - mean germination time, U_{75-25} - time between 25 and 75 % of G_{max} . For other explanations see Table 1

The percentage of seeds infested with these fungi decreased significantly, when seeds of sample I were soaked in 0.025 % *Aloe* and 0.1% *Morinda* extracts, whereas treatment with 0.05 % *Morinda* extract eradicated completely *Cladosporium* spp. from sample II seeds. In few cases applied treatments caused an increase in seed infestation with *Fusarium* spp. It was observed for sample I seeds soaked in 0.05 % *Aloe* extract and in case of sample II seeds treated with 0.025 % Huva-San TR 50 and with 0.025 % and 0.1 % *Aloe* extracts. In sample I soaking seeds in 0.025 % solution of Huva-San TR 50 increased seed infestation with *M. simplex*, whereas 0.05 % Huva-San TR 50 and 0.05 % *Aloe* extract treatments significantly decreased the presence of this fungus. In case of sample II, all

solutions of *Aloe* extract, as well as Huva-San TR 50 at concentrations of 0.025 % and 0.1 %, and *Morinda* extract at concentration 0.1 % effectively controlled *M. simplex*. *Stemphylium botryosum* was recorded on a small number of sample II seeds and none of the treatments affected its presence. On the contrary, 22 % of untreated seeds of sample I was infested with this fungus, and treatment with 0.05 % and 0.1 % Huva-San TR 50, as well as 0.025 % *Morinda* extract reduced its incidence to 9.0 %, 6.5 % and 11.0 %, respectively.

Table 3
Effects of plant extracts and Huva-San TR 50 on seed infestation with fungi (the percentage of infested seeds)

Treatments	<i>Alternaria alternata</i>	<i>Alternaria dauci</i>	<i>Alternaria radicina</i>	<i>Cladosporium spp.</i>	<i>Fusarium spp.</i>	<i>Melanospora simplex</i>	<i>Stemphylium botryosum</i>
Sample I							
C	98.0 c	0 a	37.0 c	29.5 cd	2.5 a	19.5 b	22.0 c
WC	97.5 bc	0 a	27.0 a-c	24.5 a-d	2.5 a	19.0 b	13.0 a-c
A 0.025 %	90.5 a	0 a	24.5 a-c	17.5 a	3.0 a	15.0 ab	15.0 bc
A 0.05 %	91.0 a	0.5 ab	20.5 a	28.5 c-d	35.5 b	9.5 a	14.0 bc
A 0.1 %	91.5 a	1.0 ab	32.0 a-c	19.0 a-c	4.5 a	18.0 b	14.0 bc
M 0.025 %	92.0 a	0 a	23.0 ab	27.5 a-d	3.5 a	12.0 ab	11.0 ab
M 0.05 %	93.5 ab	0 a	24.0 ab	27.0 a-d	2.5 a	15.5 ab	15.5 bc
M 0.1 %	92.5 ab	0 a	28.0 a-c	19.5 ab	5.0 a	19.0 b	13.5 a-c
HS 0.025 %	95.0 a-c	1.5 b	21.0 a	30.5 d	5.0 a	30.5 c	12.5 a-c
HS 0.05 %	94.5 a-c	1.0 ab	34.0 bc	31.0 d	3.5 a	9.5 a	9.0 ab
HS 0.1 %	91.5 ab	0 a	31.0 a-c	21.5 a-d	5.5 a	12.0 ab	6.5 a
Sample II							
C	99.5 a	-	2.5 ab	4.5 bc	22.5 a	16.5 cd	2.0 a
WC	100.0 a	-	5.0 b	2.0 a-c	21.5 a	9.5 b-d	0.5 a
A 0.025 %	100.0 a	-	1.0 a	3.5 a-c	44.5 c	5.0 ab	2.5 a
A 0.05 %	98.5 a	-	2.0 ab	3.5 bc	24.0 ab	3.5 a	0.5 a
A 0.1 %	99.0 a	-	3.0 ab	1.5 ab	34.5 bc	5.5 ab	0.5 a
M 0.025 %	99.5 a	-	2.0 ab	3.5 a-c	30.0 ab	9.0 bc	2.0 a
M 0.05 %	98.5 a	-	4.0 ab	0 a	32.0 ab	17.0 d	1.5 a
M 0.1 %	99.0 a	-	2.0 ab	0.5 ab	26.5 ab	7.0 ab	0.5 a
HS 0.025 %	99.5 a	-	3.5 ab	5.5 c	34.0 bc	4.5 ab	2.0 a
HS 0.05 %	99.5 a	-	2.5 ab	3.5 bc	26.0 ab	9.5 b-d	1.5 a
HS 0.1 %	99.0 a	-	3.5 ab	1.0 ab	22.5 ab	6.5 ab	1.5 a

Means in columns, for each sample separately, followed by the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan's test, "--" not detected. For other explanations see Table 1

Discussion

It has been found in the present experiment that effectiveness of applied treatments was significantly connected with carrot seed quality before treatment. Sample I, which was characterized by poor germination and abundant infestation with fungi, especially *A. radicina*, responded to the treatments to a greater extent than sample II, showing relatively higher germination and lower infection by pathogenic *Alternaria* spp. The similar correlation was observed previously in relation to zinnia seeds treated with hydrogen peroxide. The sample showing high seed infestation with fungi, including *Alternaria* spp., responded better to H_2O_2 treatment, than the sample free of pathogenic *Alternaria zinniae* and with a high initial germination. The treatment also negatively affected seed germination at the final count of the initially better sample [27].

The results of current experiment showed that soaking sample I seeds in the solutions of both plant extracts, in all applied concentrations, significantly limited seed infestation with *A. alternata* compared to control. Moreover, the reduction in the incidence of *A. radicina*, the important carrot seed-borne pathogen, was observed when 0.05 % solution of *Aloe* extract, and 0.025 and 0.05 % solutions of Indian mulberry extract were applied. Seed health improvement resulted in the increase in seed germination at the first and the final counts. Tylkowska [2] reported that the increase in carrot seed infestation with *A. radicina* by 1 % reduced germination at the final count by 0.3 %. Additionally, Szopinska et al. [26] frequently detected *A. alternata*, *A. dauci* and *A. radicina* on diseased seedlings in the emergence test. Therefore, control of these fungi seems to be very important for carrot seed production. The inhibitory effect of *Aloe* and Indian mulberry extracts against *A. alternata* was observed by other researchers. Bhosale et al. [20] demonstrated antifungal activity of *M. citrifolia* extract in relation to *Alternaria alternata*, *Aspergillus flavus* and *A. niger*, isolated from groundnut (*Arachis hypogaea* L.) seeds. Saks and Barkai-Golan [11] who applied *Aloe vera* gel to control postharvest fungi: *Penicillium digitatum*, *P. expansum*, *Botrytis cinerea* and *A. alternata*, revealed that the gel suppressed germination and mycelial growth of all fungi, but *P. digitatum* and *A. alternata* were the most sensitive species. Both *Aloe* and Indian mulberry extracts contain numerous antifungal compounds, among them phenolic acids and flavonoids. According to Aguilar et al. [52] the antifungal activity of these extracts can be explained by the ability of phenols to form complexes with polysaccharides and proteins of external layers of the fungal cells. In this way the function of cell walls and membranes is destabilized causing the death of the microorganism.

In vitro study of Bajwa et al. [13] showed antifungal activity of aqueous and organic *Aloe vera* shoot extracts against pathogenic *Alternaria* spp. (*A. alternata*, *A. citri*, *A. tenuissima*). Both extracts significantly inhibited growth and biomass production of all tested fungi, but the effect depended on the applied concentration (2, 4, 6, 8 %). Several authors showed that the concentration of *Aloe* and *Morinda* extracts had a considerable impact on their antimicrobial activity [11, 15, 18, 19]. For instance, Usha et al. [19] found that the growth of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* colonies decreased with the increasing concentration of *Morinda* leaf extract. Sitara et al. [15], who evaluated the effect of 0.15, 0.25 and 0.35 % *Aloe* extracts on the growth of *Aspergillus niger*, *A. flavus*, *A. alternata*, *Drechslera hawaiiensis* and *Penicillium digitatum* on PDA medium, reported that the extract at the highest concentration completely inhibited the growth of *D. hawaiiensis* and *A. alternata*. In our experiment the relation between plant extracts concentration and its effectiveness was not so obvious, in some cases the highest efficacy revealed lower concentrated plant extracts. For example, sample I seeds treated with 0.05% *Aloe* extract showed lower infestation with *A. radicina* and *Melanospora simplex* than seeds soaked in 0.1% solution of this extract. On the other hand, the limited growth of these fungi was probably the effect of excessive development of *Fusarium* spp., which competed with other fungi on those seeds. We observed in our previous studies, that soaking seeds in aqueous solutions may favour spread of infection in case of superficially located fungi, such as *Fusarium* spp. [26, 27]. Moreover, dose-dependent reaction discussed above, was observed by other authors mostly in *in vitro* assays, in which the mechanism of competition does not exist.

Numerous reports confirm antimicrobial properties of H₂O₂ [26-30]. Hydrogen peroxide at concentrations of 3, 6, 9 and 12 % effectively controlled *A. alternata*, *A. dauci*

and *A. radicina* on carrot seeds [26], as well as *A. zinniae* and *Fusarium* spp. on zinnia seeds [27]. Treating pearl millet seeds with 1 mM H₂O₂ efficiently suppressed development of downy mildew caused by *Sclerospora graminicola* Sacc. [28]. Smilanick et al. [30] reported that soaking wheat and barley grains in 3.5% H₂O₂ completely inhibited growth of *Tilletia controversa* and *T. tritici* spores. Results of Angelova et al. [53] indicate that exposure of fungal spores to H₂O₂ promoted oxidative stress evidenced by inhibition of spore germination and biomass production. Oliveira et al. [54] reported that H₂O₂ induces conidia cell death in a dose-dependent manner and through a secondary necrosis mechanism.

Wojdyla [55] found that rising the concentration of the preparation Huva-San TR 50 (a.i. H₂O₂ and silver ions) from 0.025 to 0.1 % resulted in a significant increase in its effectiveness against *Sphaerotheca pannosa* var. *rosae* (powdery mildew) on roses and *Oidium* spp. on cissus plants. In our study the biggest reduction in seed infestation with *A. radicina* was observed after treating carrot seeds with Huva-San TR 50 at the lowest concentration (0.025 %), what was connected with the increase in the incidence of *Melanospora simplex*. It seems possible that both plant extracts as well as Huva-San TR 50, applied at lower concentrations, favoured growth of non-pathogenic fungi, which competed with seed-borne pathogens.

In our experiment treatments with *M. citrifolia* fruit extract significantly improved seed germination in sample I. This beneficial effect was probably connected not only with a reduction in seed infestation with *Alternaria* spp., what we mentioned above, but also with an antioxidative activity of this extract. Indian mulberry extract contains several flavonoids (i.a. kaempferol, rutin, quercetin, narcissoide) and vitamins (i.a. ascorbic acid, tocopherol) on proven antioxidant properties [4]. *Aloe* extract, which improved seed germination at the highest concentration, also contains numerous antioxidants, such as phenolic acids and flavonoids [3].

It has been found that seed germination at the final count in sample I after Huva-San TR 50 treatments was higher by 6.3 to 9.0 % compared to untreated seeds. However, the results were not statistically proven. Hydrogen peroxide may enhance seed germination by control of seed-borne pathogens, what has been mentioned above, and additionally by oxidation of germination inhibitors present in the pericarp [26-30, 41]. In the face of the fact that applied concentrations of Huva-San TR 50 did not affect negatively seed germination, the use of higher doses of this preparation or/and prolongation of seed exposition should be considered in the future experiments.

Conclusion

1. Treating seeds of sample I with *Morinda citrifolia* plant extract at concentrations of 0.025 and 0.05 % reduced their infestation with *Alternaria alternata* and *A. radicina*, increased germination at the first and the final counts, and did not affect seed vigour.
2. The effects of *Aloe* extract and disinfectant Huva-San TR 50 on seed quality parameters varied depending on their concentration. Hence, further studies are necessary to establish the optimal conditions for carrot seed treatment.

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