NON-CHEMICAL METHODS FOR CONTROLLING SEEDBORNE FUNGI IN CARROT WITH SPECIAL REFERENCE TO *ALTERNARIA RADICINA*

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Abstract: The objective of the work was to study the efficacy of carrot seed treatment with antagonistic microorganisms *Clonostachys rosea*, *C. solani*, and grapefruit extract (Biosept 33 SL™). Seeds were artificially inoculated with *Alternaria radicina* spores prior to treatment. The presence of fungi, seed germination and vigour were determined in laboratory tests, whereas seedling emergence and health were determined in sand and soil assays. *Clonostachys* spp. and grapefruit extract significantly reduced seed infestation with *A. radicina* and improved seed germination capacity. At a lower concentration of ioculum, treatment with *Clonostachys* spp. increased percentage of healthy plants in sand assay. At a higher concentration of inoculum, treatment with Biosept 33 SL™ at 0.5% was more effective. Inoculation with pathogen and, in most cases, the following treatments, did not affect total plant emergence in a soil assay. However, pathogen affected significantly a percentage of healthy plants. Treatment with Biosept 33 SL™ at 0.5% as well as with *C. rosea* increased their numbers significantly at both concentrations of inoculum.

Key words: biological control, *Alternaria radicina*, *Clonostachys rosea*, *Clonostachys solani*, grapefruit extract, carrot seeds

INTRODUCTION

Alternaria radicina M.D. et E. [syn. *Stemphylium radicinum* (Meier, Drechsler et Eddy) Neerg.] is an important seedborne pathogen, causing seed decay and damping-off, and black root rot in carrot (*Daucus carota* L.) (Tylkowska 1992; Dorna 2007). Fungicide seed treatments especially by using iprodione can control *Alternaria* pathogens on carrot seeds efficiently, but the use of fungicides is increasingly restricted in Europe and not allowed in organic farming. Therefore, alternative seed treatments are demanded. Biological control by use of antagonistic microorganisms or plant extracts which inhibit pathogen growth are potential measures for disease control.

There are, however, only a few examples of biocontrol of seedborne *Alternaria* pathogens in carrot. Seed treatment with an isolate of *Bacillus subtilis* (FZB Biotechnik GmbH9) improved seedling emergence from seeds artificially inoculated with *A. radicina*, but mainly at temperatures above 20°C (Bochow 1992; Jamal *et al*. 1993). Chen and Wu (1999) selected nine isolates of antagonistic microorganisms after *in vitro* screening of 1 897 isolates originating from soil, carrot seeds or tap roots. The authors, using seeds artificially inoculated with *A. radicina*, stated that the efficacy of the bacterium *Burkholderia cepacia*, isolate 229, on plant emergence was as good as seed treatment with iprodione. On the other hand, Jahn and Puls (1998) found no effect on seedling emergence after treatment with *Pseudomonas* strain W 24 on carrot seed naturally infected by *Alternaria* spp. However, strain W 24 was originally selected for biocontrol efficacy against soilborne plant pathogens. Furthermore, Hermansen *et al*. (1999) found no effect on field emergence of seed naturally infested with *A. dauci* using the commercial biological control agents (BCAs) Mycostop (*Streptomyces griseoviridis* L. Anderson strain K61) and T-22 (*Trichoderma harzianum* Rifai strain KRL-AG2). These strains also were selected for biocontrol of soilborne diseases. This emphasizes that isolates selected for efficacy against soilborne pathogens are not necessarily suited for control of diseases caused by seedborne pathogens.

Clonostachys spp. proved a high efficacy against *A. radicina* in a screening program on carrot seeds (Jensen *et al*. 2004). It was also demonstrated there that bio-priming with the biocontrol strain, *C. rosea*, isolate IK726, made it possible to prime seeds infected with pathogenic *Alternaria* spp. without a risk of adverse effects on seedling establishment.

Exploitation of natural products such as essential oils, glucosinolates and plant extracts for management of plant diseases has recently gained more attention (Cop-

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ping and Menn 2000; Tripathi and Dubey 2004; Van der Wolf *et al.* 2008). The interest in replacement of synthetic fungicides with e.g. plant derived products is especially related to the biodegradability of natural products. Extracts showed antifungal activity and a documented effect was shown against plant pathogens *in situ* (Tripathi and Dubey 2004). Orlikowski (2001) found that grapefruit (*Citrus paradisi* Macfayden) extract inhibited the growth and sporulation of *Phytophthora cryptogea* and caused deformation of zoosporangia and disintegration of hyphae. It controlled also the development of *Phytophthora* foot rot of gerbera. The grapefruit extract activity from Biosept 33 SL™ showed efficacy in control of powerdy mildew and black spot of rose (Woydyła 2001). It was also reported that this preparation inhibited the growth of several fungi, isolated from cabbage, carrot and onion seeds, among them: *A. alternata*, *A. brassicicola*, *Botrytis aclada*, *B. cinerea*, *Fusarium avenaceum* and *F. oxysporum* (Dorna *et al*. 2005). Moreover, Biosept 33 SL™ effectively controlled pathogenic fungi occurring on cabbage, onion and zinnia seeds (Szopińska *et al*. 2007). In a screening program testing plant extracts against *A. radicina* in agar, Biosept 33 SL™ was among the most promising (Tylkowska, unpublished).

The objectives of the presented work were: 1) to study the effect of seed treatment with *Clonostachys* spp. and the plant extract Biosept 33 SL™ on the incidence of seedborne fungi of carrot, especially *A. radicina*, and 2) to evaluate and compare the efficacy of seed treatments on seedling establishment using three screening systems: germination test, sand and soil assays.

MATERIALS AND METHODS

Antagonists and plant extracts

The fungal isolates *C. rosea* IK1871 and *C. solani* IK1889 were both isolated from carrot seeds incubated on blotter and selected as potential biocontrol agents against *A. radicinia* in a selection program in carrot seeds (Jensen *et al*. 2004), and *C. rosea* IK726 was isolated from roots of barley (Knudsen *et al*. 1995) and selected in a selection program against *F. culmorum* in barley seeds (Knudsen *et al*. 1997).

Biosept 33 SL™ is a grapefruit based extract produced by Cintamani Poland, Piaseczno.

Seed inoculation and treatments

Seeds of carrot cv. Bolero, characterized by low (0.5%) infection with *A. radicina* were artificially inoculated by soaking in suspensions of the fungus (isolate R 25) at the concentrations of either 10^4 or 10^5 conidia/ml. Seeds were shaken (IKA Vibrax; IKA Works, Wilmington, NC) in the spore suspension at 1:10 w/v for 10 min at 130 rpm. After inoculation the seeds were dried in laminar air over night. Next day, the seeds were treated with either the biocontrol agents, Biosept 33 SL™, a fungicide or water. Seeds were soaked for 30 min in aqueous solutions of Biosept 33 SL™ at the concentration of 0.05, 0.2 and 0.5%, and surface dried. Fungal spores of *C. rosea* IK1871 and IK726 and *C. solani* IK1889 were gently scraped from PDA cultures in water and filtered through nylon mesh (38 μm). All spore-solutions were adjusted with sterile water to a density concentration of 1 x 10^7 conidia/ml. The fungi were coated on seeds according to Jensen *et al*. (2004) by shaking 1 g seeds per treatment with 4 ml of the adjusted conidial suspension for 10 min at 130 rpm. Iprodione at 5g/kg seed was the fungicide control. Water controls included treatment for 10 min as for BCAs treatments and 30 min as for Biosept 33 SL™ treatments.

Evaluation

The mycological analysis, seed germination test and seedling emergence in sand and soil were performed for: untreated seeds, seeds inoculated with *A. radicina*, inoculated seeds treated with water for 10 min and 30 min, inoculated seeds treated separately with spores of *C. rosea* IK1871, *C. rosea* IK726 and *C. solani* IK1889, inoculated seeds treated with Biosept 33 SL™ at the concentration of 0.05, 0.2 and 0.5%, and inoculated seeds treated with Iprodione at 5g/kg seeds.

Mycological analysis

These assessments were performed on 200 seeds from each treatment by using the deep-freezing-blotter method. Seeds were incubated in Petri dishes on moist blotter, 20 per dish, for three days at 20°C in darkness, then transferred to –20°C for 16 h and subsequently incubated for seven days at 20°C under alternating cycles of 12 h NUV light and 12 h darkness. The fungi were identified on the basis of growth and sporulation characteristics using a stereo-microscope and a compound microscope.

Seed germination tests

The germination test was conducted on moist blotter in Petri dishes at 20°C in darkness in four replications of 100 seeds from each treatment. The percentages of normal seedlings, abnormal seedlings (diseased seedlings) and dead seeds were determined after 7 and 14 days according to the ISTA Rules (2003). The percentage of germinated seeds and speed of germination was determined in four replications of 100 seeds from each treatment at the conditions described already. Germinating seeds (root protrusion) were scored daily and removed from the plate, until no new seed germinated. The germination rates, characterising seed vigour, i.e. T_1 – time to 1% of the total number of germinating seeds and T_{50} – time to 50% of the total number of germinating seeds, were calculated according to Jalink and Van der Schoor (1999).

Sand and soil tests

Sand bioassay followed the procedure by Jensen *et al*. (2004). Seeds were sown in washed coarse sand moistened with tap water (3:1, vol/vol) in plastic boxes (11x17 cm) with 50 seeds in each box. Each treatment was replicated four times (in total 200 seeds per treatment) and arranged in a fully randomized block design. The boxes were covered with transparent plastic bags, and incubated in a growth chamber during a 12 hours light period at 20±1°C. The seedlings were watered with tap water using a water-atomiser after 8 and 14 days.

Soil bioassay were performed in plastic containers (15x32 cm) with universal pot soil (Kronen) moistened with tap water (3:1, vol/vol) with 100 seeds in each box. Each treatment was replicated four times. Seeds were incubated under the same conditions as in the previous test.

For both tests seedling emergence and health were assessed after 7, 14 and 21 days. Numbers of healthy emerged seedlings were evaluated after 21 days.

Statistical analysis

The results obtained were evaluated by analysis of variance followed by Duncan's multiple range test at $\alpha = 0.05$.

RESULTS

Mycological analysis

Deep-freeze blotter health test revealed that untreated seeds were infested with *A. radicina* to a trace degree (0.5%), whereas *A. alternata* occurred on 87.0% of the seed (Table 1a, b). Inoculation with *A. radicina* at low and high conidia suspension densities, resulted in 44.5% and 96.0% of infested seeds, respectively. Water control treatments diminished the percentage of infested seeds, by about half at a lower inoculum density whereas to a lower degree at a higher inoculum density. All biological control agents as well as Biosept 33 SL™ significantly reduced seed infestation with *A. radicina.* BCAs *C. rosea* IK726, IK1871 and *C. solani* IK1889 as well as Biosept 33 SL™ at 0.05% were equally effective in lowering seed infestation in the less infested sample. At both infestation levels of the pathogen, efficacy of Biosept 33 SL™ increased with increasing concentration, but the differences between concentration levels were more pronounced at high infested sample compared with less infested one. Treatment with Biosept 33 SL™ at the highest degree resulted in reducing seed infestation to the level compared with that of uninoculated control. *C. rosea* IK726 and IK1871 and Biosept 33 SL™ at 0.2% diminished the percentage of infested seeds to the comparable degree. Iprodione eradicated *A. radicina* from the seeds despite of their infestation level. The naturally occurring fungi in seeds (*A. alternata*, *Cladosporium* spp., *Epicoccum purpurascens* and *Stemphylium botryosum*) showed to be at the same level on unioculated as inoculated seeds at both concentrations except for *Fusarium* spp. Inoculation of seeds with *A. radicina* favoured the growth of these fungi. However, water treatment diminished the *Fusarium* density and the antagonists as well as Biosept 33 SL™ further decreased this level.

Neither seed inoculation with *A. radicina* nor the water and BCAs treatments affected numbers of seeds free from fungi. However, the parameter increased significantly as the seeds were treated with Iprodione, and to a lesser extent, as the seeds were treated with Biosept 33 SL™ preparation at 0.2 and 0.5%, regardless of inoculum concentration.

Table 1. Effect of seed treatment with antagonists and plant extract preparation on the incidence of fungi

a. Seeds inoculated with *A. radicina* at inoculum density of 10^4 conidia/ml

Treatment	Seeds infested with [%]						Seeds free from fungi [%]
	A. radicina	*A. alternata*	*Cladosporium* spp.	*E. purpurascens*	*Fusarium* spp.	*S. botryosum*	
Control*	0.5 e**	87.0 a–c	90.0 a	7.0 ab	3.0 cd	19.5 a	0.5 d
i + control	44.5 a	91.5 ab	75.5 bc	8.0 a	47.0 a	13.5 ab	0 d
i + water 10 min	19.5 b	91.5 a	90.0 a	10.5 a	4.0 c	12.5 a–c	1.0 d
i + water 30 min	24.5 b	81.5 b–d	91.0 a	7.5 ab	16.5 b	12.5 a–c	0.5 d
i + IK726	6.0 c	74.0 cd	77.0 bc	6.5 ab	4.5 c	10.0 a–c	0.5 d
i + IK1871	7.0 c	63.5 ef	72.0 bc	4.5 ab	1.0 ef	8.5 bc	0 d
i + IK1889	7.0 c	71.5 de	83.0 ab	2.5 bc	0 f	10.5 a–c	0 d
i + Biosept 0.05%	8.5 c	87.5 a–c	64.5 c	6.0 ab	2.0 de	13.5 ab	0 d
i + Biosept 0.2%	3.0 d	75.0 de	45.0 d	1.0 cd	0 f	10.0 a–c	9.5 c
i + Biosept 0.5%	0 e	49.5 f	12.5 f	1.0 cd	0 f	6.5 cd	38.5 b
i + Iprodione	0 e	3.0 g	26.0 e	0 d	2.5 c–e	2.5 d	68.0 a

*control – untreated seeds; i + control – seeds inoculated with *A. radicina*; i + water 10 min – inoculated seeds treated with water for 10 min; i + water 30 min – inoculated seeds treated with water for 30 min; i + IK1871 – inoculated seeds treated with spores of *C. rosea* IK1871; i + IK726 – inoculated seeds treated with spores of *C. rosea* IK726; i + IK1889 – inoculated seeds treated with spores of *C. solani* IK1889; i + Biosept 0.05% – inoculated seeds treated with Biosept 33 SL™ at concentration of 0.05%; i + Biosept 0.2% – inoculated seeds treated with Biosept 33 SL™ at concentration of 0.2%; i + Biosept 0.5% – inoculated seeds treated with Biosept 33 SL™ at concentration of 0.5%; i + Iprodione – and inoculated seeds treated with Iprodione at 5 g/kg seeds

**values in columns followed with the same letter are not significantly ($\alpha = 0.05$) different from each other

b. Seeds inoculated with *A. radicina* at inoculum density of 10^5 conidia/ml

Treatment	Seeds infested with [%]						Seeds free from fungi [%]
	A. radicina	*A. alternata*	*Cladosporium* spp.	*E. purpurascens*	*Fusarium* spp.	*S. botryosum*	
Control	0.5 gh	87.0 a	90.0 a	7.0 a	3.0 b–d	19.5 a	0.5 c
i + control	96.0 a	83.0 a	88.0 a	7.0 a	34.0 a	4.5 c	0 c
i + water 10 min	85.5 b	85.5 a	87.5 a	6.5 a	5.0 b	7.5 bc	0 c
i + water 30 min	67.5 d	83.0 a	86.0 a	5.5 a	1.0 de	9.0 bc	0 c
i + IK726	17.0 f	69.5 de	83.0 ab	5.0 a	1.0 d	7.5 bc	0.5 c
i + IK1871	14.5 f	70.0 b	71.0 cd	4.0 ab	1.0 de	6.5 bc	0 c
i + IK1889	28.0 e	69.5 b	76.5 bc	4.0 ab	0.5 d	9.0 bc	0.5 c
i + Biosept 0.05%	78.0 c	79.5 ab	62.0 d	7.5 a	8.0 b	12.5 ab	0 c
i + Biosept 0.2%	11.5 f	56.5 c	22.5 ef	0.5 bc	1.5 c–e	8.5 bc	30.5 b
i + Biosept 0.5%	2.0 g	56.0 c	15.0 f	0.5 bc	1.0 de	7.0 bc	32.5 b
i + Iprodione	0 h	3.5 d	28.0 e	0 c	4.0 bc	3.5 c	65.0 a

Explanation – see table 1a

Seed germination

Evaluation of germination characteristics in blotter test showed that inoculation with *A. radicina* did not affect a percentage of germinating seeds, whereas it significantly decreased germination capacity, primarily due to the increase in percentage of diseased seedlings (Table 2a, b). This was more pronounced at a higher level of inoculum density. All but one treatments (*C. solani* IK1889) following inoculation with *A. radicina* at the low concentration significantly improved germination capacity compared not only with inoculated but also with the uninoculated control (Table 2a). From inoculation with *A. radicina* at the high concentration, all treatments resulted in a germination capacity at level of or better than uninoculated control. Biosept 33 SL™ at 0.2% and 0.5% and, *C. rosea* IK726 resulted in the highest increase in germination capacity not statistically different from Iprodione (Table 2b).

A dramatic increase in a number of diseased seedlings was observed after seed inoculation with *A. radicina*, especially at a higher inoculation level. All treatments reduced the numbers significantly compared to inoculated control and in a range comparable or better than uninoculated untreated control.

Application of *A. radicina* at a higher inoculum density resulted in the increase in percentage of dead seeds (Table 2b). Treatment with Iprodione diminished their occurrence to the initial level. At both inoculum densities, application of either BCAs or Biosept 33 SL™ did not cause visible effects on percentages of these seeds.

Regardless of inoculum density neither seed inoculation with *A. radicina* nor the following treatments affected T_1 parameters. Accelerated germination, expressed as decrease of T_{50} value, was observed after seed inoculation at a lower concentration of the pathogen (Table 3).

Table 2. Effect of seed treatment with antagonists and grapefruit extract on seed germination

a. Seeds inoculated with A. *radicina* at inoculum density of 10^4 conidia/ml

Treatment	Germinating seeds [%]	Normal seedlings [%]	Diseased seedlings [%]	Dead seeds [%]
Control	92.5 c	61.0 b	36.5 b	0.5 d
i + control	93.8 a–c	39.8 c	56.3 a	1.3 cd
i + water 10 min	96.0 a–c	62.8 b	33.3 bc	1.8 b–d
i + water 30 min	96.3 ab	65.5 b	29.0 c	4.3 ab
i + IK726	94.8 a–c	73.5 a	19.0 d–f	4.5 a
i + IK1871	94.3 a–c	77.0 a	17.8 ef	3.5 a–c
i + IK1889	96.8 ab	62.8 b	29.8 c	4.3 ab
i + Biosept 0.05%	95.3 a–c	72.5 a	23.3 d	2.5 a–d
i + Biosept 0.2%	93.0 bc	77.8 a	15.5 f	4.0 ab
i + Biosept 0.5%	95.3 a–c	78.3 a	15.5 f	2.3 a–d
i + Iprodione	97.0 a	72.8 a	22.5 de	2.3 a–d

Explanation – see table 1a

b. Seeds inoculated with A. *radicina* at inoculum density of 10^5 conidia/ml

Treatment	Germinating seeds [%]	Normal seedlings [%]	Diseased seedlings [%]	Dead seeds [%]
Control	92.5 c	61.0 d	36.5 d	0.5 e
i + control	94.0 a–c	0.8 g	94.5 a	4.5 ab
i + water 10 min	93.5 bc	11.5 f	84.5 b	4.0 a–c
i + water 30 min	96.8 a	27.3 e	68.0 c	3.3 a–d
i + IK726	94.8 a–c	72.5 a–c	21.3 gh	4.5 a–c
i + IK1871	96.0 a–c	65.8 cd	29.3 d–f	1.8 c–e
i + IK1889	96.0 a–c	65.8 cd	27.5 e–g	5.0 ab
i + Biosept 0.05%	95.0 a–c	60.5 d	32.5 de	5.8 a
i + Biosept 0.2%	96.5 a–c	77.3 a	16.0 h	4.0 ab
i + Biosept 0.5%	95.0 a–c	69.3 bc	25.0 fg	2.8 b–d
i + Iprodione	96.5 ab	75.3 ab	18.0 h	1.8 de

Explanation – see table 1a

Table 3. Effect of seed treatment with antagonists, grapefruit extract and Iprodione on speed of seed germination (days)

Treatment	Inoculum density 10^4 conidia/ml		Inoculum density 10^5 conidia/ml	
	T_1*	T_{50}	T_1	T_{50}
Control	1.27 ab	2.30 de	1.27 ab	2.30 cd
i + control	1.25 ab	2.14 a	1.31 ab	2.23 bc
i + water 10 min	1.46 b	2.22 a–d	1.63 b	2.12 ab
i + water 30 min	1.50 ab	2.13 a	1.44 ab	2.14 ab
i + IK726	1.16 a	2.29 c–e	1.38 ab	2.18 a–c
i + IK1871	1.17 ab	2.27 b–e	1.07 a	2.23 b–c
i + IK1889	1.24 ab	2.19 a–c	1.16 ab	2.12 a
i + Biosept 0.05%	1.44 ab	2.19 a–d	1.41 ab	2.16 ab
i + Biosept 0.2%	1.39 ab	2.18 a–d	1.15 ab	2.16 ab
i + Biosept 0.5%	1.38 ab	2.16 ab	1.58 ab	2.11 ab
i + Iprodione	1.40 ab	2.35 e	1.37 ab	2.36 d

*T_1 – time to 1% of the total number of germinating seeds; T_{50} – time to 50% of the total number of germinating seeds
Other explanations – see table 1a

Sand and soil assay

In sand assay, percentage values of total emergence and final healthy plant stand decreased significantly after seed inoculation with *A. radicina*, especially at a higher inoculum density (Table 4). At a lower seed infestation level, treatment with Iprodione and *Clonostachys* spp. increased percentage of healthy plants to the level of uninoculated control. This was followed by Biosept 33 SL™ at 0.05%. At a higher seed infestation level, treatment with Iprodione and Biosept at 0.5% gave the best results. These were followed by the BCAs. Biosept 33 SL™ at 0.2 and 0.05% were ineffective.

Inoculation with *A. radicina* and, in most cases, the following treatments, did not affect total plant emergence in a soil bioassay, irrespective of the inoculum density (Table 5). However, percentages of healthy plants diminished significantly after inoculation with the pathogen, especially at a higher inoculum level. Treatment at both inoculation levels with Biosept 33 SL™ at 0.5% as well as with *C. rosea* IK726 and IK1871 increased their numbers to the levels for untreated controls and Iprodione treatment.

Table 4. Effects of seed treatment with antagonists, grapefruit extract and Iprodione on seedling emergence in sand [%]

Treatment	Inoculum density 10^4 conidia/ml		Inoculum density 10^5 conidia/ml	
	total emergence	final healthy plant stand	total emergence	final healthy plant stand
Control	94.5 a	94.5 a	92.0 a	92.0 a
i + control	46.0 c	19.5 d	27.0 f	2.0 e
i + water 10 min	70.5 b	36.5 c	39.5 ef	9.0 d
i + water 30 min	67.0 b	39.5 c	40.5 ef	13.0 d
i + IK726	91.0 a	89.0 a	80.5 bc	65.5 bc
i + IK1871	93.0 a	90.0 a	73.0 c	67.5 bc
i + IK1889	90.5 a	85.5 a	79.5 bc	61.5 c
i + Biosept 0.05%	76.5 b	29.5 cd	53.5 ed	15.5 d
i + Biosept 0.2%	67.5 b	46.0 c	58.0 d	12.5 d
i + Biosept 0.5%	69.5 b	69.0 b	90.5 a-c	76.5 b
i + Iprodione	93.5 a	93.0 a	90.5 ab	88.5 a

Explanation – see table 1a

Table 5. Effect of seed treatment with antagonists, grapefruit extract and Iprodione on seedling emergence in soil [%]

Treatment	Inoculum density 10^4 conidia/ml		Inoculum density 10^5 conidia/ml	
	total emergence	final healthy plant stand	total emergence	final healthy plant stand
Control	92.5 b	90.5 ab	92.5 a–c	90.5 ab
i + control	91.5 b	83.3 c	92.5 a–c	57.0 f
i + water 10 min	92.3 b	88.0 bc	90.3 bc	63.3 ef
i + water 30 min	93.5 b	90.8 ab	94.8 a	81.3 cd
i + IK726	91.5 b	89.0 a–c	93.0 a–c	88.3 a–c
i + IK1871	93.5 b	90.0 bc	94.3 ab	89.8 ab
i + IK1889	92.5 b	88.5 a–c	94.3 ab	85.8 b–d
i + Biosept 0.05%	93.8 b	90.0 ab	93.8 ab	69.8 e
i + Biosept 0.2%	94.5 ab	92.0 ab	89.3 c	79.0 d
i + Biosept 0.5%	94.8 ab	92.8 ab	95.0 a	93.8 a
i + Iprodione	97.0 a	93.3 a	93.5 ab	91.5 a

Explanation – see table 1a

DISCUSSION

Seeds are commonly treated with chemicals. The use of biological control agents and plant origin preparations, instead of fungicides, to control *A. radicina* seems an attractive alternative, especially for organic farming, because natural source of origin allow to presuming their low harmfulness for environment.

The efficacy of *C. rosea* against different pathogens was proven earlier (Jensen *et al*. 2000; Krauss and Soberanis 2001; Morandi *et al*. 2003; Jensen *et al*. 2004; Yohalem *et al*. 2004; Nobre *et al*. 2005). These reports found a confirmation in the presented experiment. All tested *Clonostachys* spp. isolates effectively suppressed the growth of *A. radicina*, regardless of screening system and inoculum density. The treatment of plants with BCAs has in some cases resulted in enhanced resistance against *Alternaria* pathogens (Ton *et al*. 2002; Morita *et al*. 2003). Moreover, there is some evidence that biocontrol fungi such as *C. rosea* can enter the host tissues and reside as an endophyte, subsequently colonizing the tissues or potentially inducing host defenses to reduce further development of the pathogen (Sutton *et al*. 2002).

In our experiment neither Biosept 33 SL™ nor antagonists affected seed germination. Karsznicka and Grzesik (2001) found that treatment of China aster (*Callistephus chinensis* Nees.) seeds with grapefruit extract accelerated plant growth as well as greatly improved seedling uniformity, when compared to control. Growth promoting effects of IK726 were demonstrated in seedlings of barley infected with *F. culmorum* and in seedlings of carrot under field conditions (Jensen *et al*. 2000; Jensen *et al*. 2004). In the experiment of Johansen *et al*. (2005) the strain seemed to have general stimulating effects on soil enzyme activity and the soil microbiota, and resulted in a significant

increase in wheat and sugar beet plants dry weight. Furthermore, the relatively high plant growth promoting effect of *C. rosea* IK726 was reported by Ravnskov *et al*. (2006). These authors coincided tomato growth promotion, after inoculation of soil with the antagonist, with an increase in plant phosphorus content, suggesting that the fungus increased phosphorus solubility.

In the view of presented study, *C. solani* IK1889 seems to be a new promising biological control agent, especially for treating medium quality carrot seeds, comparable in effectiveness with *C. rosea* IK726 and IK1871. The isolation from carrot habitat (Jensen *et al*. 2004) increase probability that this antagonist will be able to functioning the same environmental niche as that of the pathogen it is to control.

According to the results presented above, carrot black rot caused by *A. radicina* can be effectively controlled by selected antagonists, as well as by grapefruit extract. The efficacy of the latter was even greater at a higher inoculum level, especially in sand and soil assays. Probably the mechanism of its action is similar to a fungicide and because of that less dependent on different external conditions. Activity of BCAs could be affected by several factors. Applied at high density, isolates of indigenous *Penicillium* sp. and *A. alternata* from rose (*Rosa hybrida* L.) interacted with *C. rosea* and reduced control of the pathogen by 16% and 21%, respectively (Morandi *et al*. 2000). The study of Yohalem *et al*. (2004) showed that *C. rosea* suppressed sporulation of *B. aclada* with variable efficacy. *C. rosea* was effective in suppressing *B. aclada* sporulation under constant moist conditions, but it was less effective if exposed to a drying period.

On the other hand, in the presented study, *Clonostachys* spp. was shown to be more specific against *A. radicina* compared to grapefruit extract that was found to be effective against most fungi. That resulted probably from compound chemical content of the latter. Benzethonium chloride, methyl parabene and triclosan, obtained from grapefruit seeds, suppressed development of 6 species of bacteria (Woedtke *et al*. 1999). Another compound – 7-geranoxycoumarin – isolated from flavedo tissue of grapefruit 'Star Ruby' exhibited strong antifungal activity against *Penicillium italicum* and *P. digitatum* during *in vitro* and *in vivo* tests (Angioni *et al*. 1998). This broad antifungal and antibacterial action of grapefruit extract was confirmed by some researchers (Woydyła 2001; Dorna *et al*. 2004; Dorna *et al.* 2005; Szopińska *et al*. 2007; Van der Wolf *et al.* 2008). However, there were limited reports on the potential role of this extract against seedborne fungi, because it was developed rather for foliar application. Dorna *et al*. (2005) found that grapefruit extract inhibited the growth of *A. alternata*, *A. dauci* and *A. radicina*, isolated from carrot seeds as effectively as Iprodione. Similarly, the extract suppressed the growth of most of the fungi isolated from onion and cabbage seeds. Szopińska *et al*. (2007) on the reported efficacy of grapefruit extract against *A. zinniae* on zinnia seeds and *B. aclada*, *B. cinerea* and *Fusarium* spp. on onion seeds. Van der Wolf *et al.* (2008) observed a high action of grapefruit extract against the seedborne *Xanthomonas campestris* pv. *campestris*, *Clavibacter michiganensis* subsp. *michiganensis*, *A. dauci* and *B. aclada*.

Moreover, grapefruit extract can also act as a scavenger of free oxygen radicles, which can be components of host defense against pathogen penetration (Karsznicka and Grzesik 2001).

At the presented study an accelerated germination was observed if the seeds were inoculated with *A. radicina*. This phenomenon was most probably caused by some enzymes produced by the pathogen. Many of the fungi infested seeds, among them *Alternaria* spp., are known as cellulose decomposers (Pugh 1973). Their metabolites are able to decompose pericarp of seeds and in this way influence their germination.

It is difficult to explain why *Fusarium* spp. was enhanced by inoculation of seeds with *A. radicina*, since both fungi were recognised rather as antagonists (Czyżewska 1985). Presumably the phenomenon resulted somehow from the method of seed inoculation.

Blotter test showed that inoculation with *A. radicina* did not affect the percentage of germinating seeds, whereas it significantly decreased germination capacity, primarily due to the increase in percentage of diseased seedlings. This observation was confirmed by Dorna (2007), who tested the effect of inoculation of *A. radicina* on carrot seed germination. Tylkowska (1992) reported that increase in seed infection by this pathogen resulted in the reduction of germination capacity of carrots. On the other hand, final plants stand in the soil, especially at lower inoculation level, did not correspond well with the results of germination test. It seems purposeful to complete the standard seed germination test recommended by ISTA (International Seed Testing Association) with additional seed health analysis to obtain reliable prediction of healthy plant stand.

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POLISH SUMMARY

NIECHEMICZNE METODY ZWALCZANIA GRZYBÓW ZASIEDLAJĄCYCH NASIONA MARCHWI ZE SZCZEGÓLNYM UWZGLĘDNIENIEM *ALTERNARIA RADICINA*

Celem pracy było zbadanie efektywności zaprawiania mikroorganizmami antagonistycznymi *Clonostachys rosea*, *C. solani* i ekstraktem z grejpfruta (Biosept 33 SL™) nasion marchwi, sztucznie inokulowanych zarodnikami grzyba *Alternaria radicina*. W testach laboratoryjnych oceniano zasiedlenie nasion przez grzyby, kiełkowanie i wigor, natomiast wschody siewek i ich zdrowotność badano w piasku i uniwersalnym podłożu do wysiewu nasion. Grzyby rodzaju *Clonostachys*, podobnie jak ekstrakt z grejpfruta, znacząco ograniczyły zasiedlenie nasion przez *A. radicina* i poprawiały zdolność kiełkowania nasion. Przy mniejszym stężeniu inokulum zaprawianie nasion *Clonostachys* spp. zwiększało wschody i udział zdrowych roślin w piasku. Przy większym stężeniu inokulum, bardziej efektywne było zaprawianie preparatem Biosept 33 SL™ o stężeniu 0,5%. Inokulacja nasion patogenem i w większości przypadków zaprawianie, nie miały wpływu na wschody roślin w podłożu do wysiewu nasion, ale patogen wpływał istotnie na ich zdrowotność. Niezależnie od stężenia inokulum zaprawianie nasion preparatem Biosept 33 SL™ w stężeniu 0,5% oraz grzybem *C. rosea*, znacząco zwiększało liczbę zdrowych roślin.