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Potential pathogens of common caraway (*Carum carvi* L.) seeds and search for measures suppressing their spread

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Abstract

The potential pathogens of cultivated and wild common caraway (*Carum carvi* L.) seeds of 2001–2004 harvest were studied. Ripe seeds were collected in various localities of Biržai, Kaunas, Raseiniai, Šilutė, Ukmergė, Varėna and Vilnius districts in June–July.

The fungi of 18 species and 14 genera attributable to potential pathogens, the agents of plant spots, wilts and rots, were identified in common caraway seeds. They were detected in almost all seed samples each year and their frequency of occurrence amounted to up to 44.0% in total. The fungi of *Alternaria* genus prevailed and their isolates accounted for 86.9% of the total amount of potential pathogens. Among other potential pathogens *Phoma* spp., *Phomopsis diachenii*, *Stemphylium botryosum*, *Ascochyta biforae*, *Fusarium avenaceum*, *Botrytis cinerea* were detected more frequently. Although the occurrence of some potential pathogens was not very high, in the individual seed samples their incidence was quite high and could be important for the spread of diseases in caraway crops. The potential pathogens ascertained in the seeds of cultivated and wild caraway had very high qualitative and quantitative similarity; however, they differed significantly between years and localities.

Aggressive strains (*Fusarium avenaceum* 11212 and *Phoma anethi* 11201) were ascertained in pathogenicity test of fungi, detected in common caraway seeds. They affected caraway seedlings *in vitro*. The common caraway variety 'Kančevitskij' was the most resistant to these pathogens. In pot trials, infected plants exhibited a poorer over-winter survival and produced seeds of lower quality.

Because the application of chemical measures in common caraway crops is problematic, the use of resistant varieties, accumulating higher contents of essential oils and antagonistic bacteria might be an advisable alternative for the control of seed-borne pathogens.

Key words: *Carum carvi*, seeds, fungi, pathogens, occurrence, pathogenicity, resistance, control.

Introduction

Increased interest in healthy diet and natural products has expanded the demand for medicinal and spice plants (Gabler, 2002). Their growing and application in pharmaceutical, food, cosmetics and other processing industries are among the most significant developing fields in the world recently. Increasing extent of medicinal and spice plants' cultivation resulted in concentration of their crops and rising number of pathogens, which can cause considerable economic losses in yield and its quality. Seed health, which depends on the presence or absence of disease-causing organisms, is an important factor for the successful cultivation of crops. The fungi form a major group among seed pathogens. They are seed-borne as well as seed-transmitted and are frequently responsible for the spread of diseases in crops, including caraway.

In recent years, diseases of common caraway are being investigated more extensively. Various pathogens were described in neighbouring countries. It is indicated that anthracnose (the agent *Mycocentrospora acerina* (Hart.) Deighton) is widespread in the Netherlands (Evenhius, 1998), septoriosis (the agent *Septoria carvi* Syd.) in Poland (Zalewska, Machowicz-Stefaniak, 2003; Zalewska, 2008) and Austria (Bedlan, 2005). This pathogen was observed in common caraway crops in Czech Republic and Germany as well (Ondřej, 1983; Gabler, Machowicz-Stefaniak, 2004). *Phomopsis diachenii* Sacc. was identified in 1998 in Germany (Gabler, Ehrig, 2000; Gabler, 2001), later in Bulgaria (Rodeva, Gabler, 2004), Hungary (Nagy, 2009), Poland (Machowicz-Stefaniak, 2009). Other common caraway disease agents are as follows: *Alternaria alternata* (Fr.) Keissl., *A. tenuissima* (Kunze) Wiltshire, *Ascochyta carvi* Ondřej, *Botrytis cinerea* Pers., *Colletotrichum dematium* (Fr.) Grove, *C. gloeosporioides* (Penz.) Penz. & Sacc., *Erysiphe heracley* DC, *Phoma exigua* var. *exigua* Desm., *Ulocladium consortiale* (Thüm) E.G. Simons, *Fusarium* spp., *Rhizoctonia solani* J.G. Kühn, *Sclerotinia sclerotiorum*

(Lib.) de Bary (Mazur, Nawrocki, 2004; Machowicz-Stefaniak, Zalewska, 2008; Zalewska, 2010). In Lithuania, diseases of common caraway have not been studied yet. It has only been noted that *Sclerotinia* and *Fusarium* root rot are widespread in caraway crops and no description of the disease agents and indication of their species have been given (Dastikaitė, 1997). Many of the mentioned common caraway pathogens are found in seeds and can be seed-transmitted (Duczek, Slinkard, 2003; Mazur, Nawrocki, 2004; Odstričilová, 2007).

The objective of the present study was to ascertain the extent of potential pathogens' infection on common caraway seeds and to search for measures suppressing their growth.

Materials and methods

Evaluation of fungal infection. The seeds of cultivated ('Gintaras', 'Kančevitskij', 'Rekord') and wild common caraway (*Carum carvi* L.) of the 2001–2004 harvest were analysed in the Laboratory of Phytopathogenic Microorganisms, Lithuanian Institute of Botany. Ripe seeds were sampled in various localities of Biržai, Kaunas, Raseiniai, Šilutė, Ukmergė, Varėna and Vilnius districts in June–July. A total of 21 seed samples were tested. The blotter and pure culture methods were applied for the detection of fungi on and inside the seeds (Mathur, Kongsdal, 2003; Mačkinaitė, 2010; 2011). Fungi developed from each seed were estimated and identified based on their morphological and cultural characteristics according to the different manuals for fungi identification. The frequency of occurrence (FO) of separate species (percent ratio of number of seeds where the species was detected to the total number of tested seeds) and the species relative density (RD) (percent ratio of particular species isolate number to the total number of isolates) in the tested common caraway seeds were calculated (González et al., 1995). The FO indicates the distribution of a particular species in a tested object as the RD denotes its abundance against other fungi species detected there. The qualitative and quantitative similarity of potential pathogens, ascertained in the seeds of cultivated and wild caraway as well as in the seeds of different harvest years and localities was compared by calculating Sorenson's index (SI). The similarity of fungal complexes is small if SI amounts to 39.0%; moderate if SI makes up from 40.0% to 49.0%; high if SI makes up from 50.0% to 59.0%; and very high if SI amounts to more than 60.0% (Maguran, 1988). The classification of fungi was based on "Dictionary of Fungi" (Kirk et al., 2008) and Index Fungorum (http://www.indexfungorum.org). The pure isolates have been deposited in the collection (BILAS) of Institute of Botany, Nature Research Centre, Vilnius, Lithuania.

Evaluation of the pathogenicity. The pathogenicity of fungi, detected in common caraway seeds, was tested on *C. carvi* 'Kančevitskij' seedlings *in vitro* (Blok, 1997). Seven strains of the most frequent potential pathogens were selected for study: *Alternaria alternata* 11214, *Fusarium avenaceum* 11212, 11219, 11220, *F. sambucinum* 11226, *F. sporotrichioides* 11222, and *Phoma anethi* 11201. The single spore cultures of tested pathogens were plated in Petri dishes on malt extract agar (MEA) and incubated for 20 days at 24ºC in darkness. The seeds of *C. carvi* 'Kančevitskij' were sterilized with 3% NaOCl for 1 min, rinsed with sterile water three times and drained with sterile filter paper. The surface sterile seeds were plated in Petri dishes on 2% water agar (WA) and incubated at 24ºC. Evenly sprouted seedlings (about 1 cm in length) were inoculated with mycelium of the tested fungus and planted in sterile test tubes with Knoop's nutrient solution agar (one seeding per tube). In addition, each seedling was inoculated by placing a piece of tested fungus mycelium on the seedling root. The tubes were placed in climatic chamber with 16 h light and 8 h darkness period and constant 26ºC temperature. The pathogenicity of the tested fungi was evaluated after 14 and 30 days according to the seedling length and the amount of healthy, injured and dead seedlings. The experiment was carried out in five replications.

Evaluation of resistance. The resistance of common caraway to the potential pathogens was studied in pot experiment. The seeds of *C. carvi* 'Gintaras', 'Kančevitskij' and 'Record' were sterilized with 3% NaOCl for 1 min, rinsed with sterile water three times and sprouted in Petri dishes on 2% WA at 24ºC temperature. The single spore cultures of *Fusarium avenaceum* 11212 and *Phoma anethi* 11201, which demonstrated the highest aggressiveness *in vitro* test, were plated in Petri dishes on MEA and incubated for 15 days at 24ºC in darkness. The pots were filled with sterile soil, infected with pure culture of tested pathogen. One Petri dish per pot was taken. Ten seedlings with 1-cm long roots were planted per pot. In the control treatment the seedlings were grown in an infection-free soil. The experiment was carried out in three replications. The resistance of the tested common caraway varieties was evaluated according to the amount of healthy plants after 30 days and over-winter as well as in the end of the first and second vegetation periods.

The quality of matured seeds (germination, germinating power, 1000 seed weight) was assessed in accordance with the standards and methods of seed quality evaluation (Mathur, Kongsdal, 2003). The investigation was carried out in four replications with 100 seeds per replication.

The study of fungicides and caraway essential oil impact on potential pathogens' growth in vitro. The disk-diffusion method was applied for the study (Klement et al., 1990). The impact of nine chemical agents: Dithane M-45 (mancozeb 800 g kg^{-1}), Zato 50 WG (trifloxystrobin 500 g kg^{-1}), Signum 334 WG (boscalid 267 g kg^{-1} + pyraclostrobin 67 g kg^{-1}), Efector (dithianon 700 g kg^{-1}), Euparen Multi 500 WG (tolylfluanid 500 g kg^{-1}), Kemikar T (carboxin 200 g kg^{-1} + thiram 200 g kg^{-1}), Cruiser OSR (tiamethoxam 280 g l^{-1} + fludijoxonil 8.0 g l^{-1} + matalaxil-M 33.3 g l^{-1}), Maxim 025 FS (fludijoxonil 25 g l^{-1}), Previcur 607 SL (propamocarb hydrochloride 607 g l^{-1}) and stream-distilled undiluted (100%) caraway essen-

tial oil on *in vitro* growth of fungi, frequently detected in *C. carvi* seeds, was tested. Eight strains of fungi, isolated from common caraway seeds, collected in various localities, were selected: *Alternaria alternata* 11215, 11331, *A. radicina* 11332, *Fusarium avenaceum* 11212, 11335, *F. sporotrichioides* 11337, *Phoma anethi* 11201, and *Ulocladium oudemansii* 11334. The powder of tested chemical was mixed with sterile distilled water to achieve 0.1% concentration. The sterile filter paper discs were soaked with tested chemical or essential oil and arranged (three discs per Petri dish) around the tested single spore 3-day old fungus colony on MEA. The control involved sterile discs soaked with sterile distilled water. Petri dishes were incubated at constant 24ºC temperature in the dark. The experiment was carried out in three replications. The impact of the tested chemical preparation and caraway essential oil on fungus growth was evaluated after 3, 10 and 20 days according to the mycelium growth rate and nature. The mycelium growth inhibition of the tested fungus was estimated according to the formula:

$CGI = [(D_c - D_e\}/D_c] \times 100$ %, where CGI – fungus colony growth inhibition (%), D_c – diameter of fungus colony in the control, D_e – diameter of fungus colony in the experiment.

The study of bacteria impact on fungi growth in vitro. The impact of five strains (No. 1128, 1132, 1134, 1136, 506) saprotrofic *Erwinia* genus bacteria, isolated from common caraway seeds, on the growth *in vitro* of fungi (*Alternaria alternata* 11215, *Fusarium avenaceum* 11212, *Phoma anethi* 11201), widespread in *C. carvi* seeds, was studied. The interaction of microorganisms was tested *in vitro* in dual-plate assay on MEA (Logrieco et al., 1994). The plates were incubated at constant 26ºC temperature in the dark. The impact of bacteria was evaluated after 7, 10 and 25 days. The experiment was carried out in three replications.

Statistical analysis. The *SPSS 17* statistical package was used to analyze the obtained data. The significance of correlation and mean differences as well as standard error were calculated in the analyses of pathogenicity, resistance and fungicide or caraway oil impact on fungi growth.

Results and discussion

The research showed that common caraway seeds are heavily contaminated with fungi. Micromycetes of 78 species, two varieties and 54 genera, belonging to *Ascomycota*, *Basidiomycota* and *Zygomycota* phyla, *Sordariomycetes*, *Dothideomycetes*, *Leotiomycetes*, *Eurotiomycetes*, *Sacharomycetes*, *Agaricomycetes* and *Incertae sedis* classes were detected and identified inside and on the investigated common caraway seeds. Fungi attributable to potential pathogens, the agents of plant spots, wilts and rots, belong to 18 species and 14 genera (Table 1). They were detected in almost all seed samples each year and their frequency of occurrence amounted to up to 44.0% in total.

Fungi of *Alternaria* genus prevailed among the potential pathogens and their isolates accounted for 39.84% of the total isolate amount. The RD of potential pathogens from other genera amounted to 0.02–1.21% (Fig. 1).

Four potential pathogenic species of *Alternaria* genus were detected in common caraway seeds. They made up 86.9% of the total isolate amount of potential pathogens. *Alternaria alternata* and *A. radicina* prevailed among them (RD 71.2% and 14.1% among potential pathogens, accordingly) (Fig. 2). *Phoma* spp. (RD 2.6 %), *Phomopsis diachenii* and *Stemphylium botryosum* (RD 1.8%), *Ascochyta biforae* and *Fusarium avenaceum* (RD 1.7%), *Botrytis cinerea* (RD 1.1%) could be ascribed to more frequent ones among the potential pathogens. The RD of other potential pathogens identified in common caraway seeds did not exceed one percent and accounted for 2.8% (Fig. 2).

The occurrence of fungi as well as potential pathogens in common caraway seeds depended on the harvest year and sampling locality (Mačkinaitė, 2010; 2011).

The potential pathogens in infected seeds of cultivated and wild caraway had very high qualitative and quantitative similarity (Sorenson's indices (SI) 68.4% and 92.2%, accordingly), however they significantly differed between years and localities. The comparison of potential pathogens' similarity in common caraway seed samples, collected in various localities showed that 46.2% and 35.2% of them had very high and high qualitative and quantitative similarity, accordingly (SI ranged between 50.0–100.0%). While 22.0% and 26.4% seed samples of wild caraway and 33.3% and 19.0% seed samples of cultivated caraway, accordingly, show very high qualitative and quantitative similarity. The higher qualitative similarity of potential pathogens, both in the wild and cultivated common caraway seed samples, collected in different localities, was ascertained.

The study of pathogenicity *in vitro* of five fungi species, occurring in common caraway seeds, showed that all seven strains tested are pathogenic to *C. carvi* 'Kančevitskij' seedlings (Table 2). The most aggressive among them were *F. avenaceum* 11212, 11220 and *P. anethi* 11201. The length of common caraway seedlings infected with *F. avenaceum* 11212 reached only 50.3% of that of the control seedlings and they all died after 14 days' cultivation. Slightly less aggressive were *F. avenaceum* 11220 and *P. anethi* 11201. The length of the seedlings infected with these pathogens after 30 days of growing reached 66.1% and 37.8% of that of infection-free seedlings. 40.0% of seedlings died and 60.0% wilted after infection with *F. avenaceum* 11220, and 80.0% of seedlings were killed and only 20.0% remained healthy after infection with *P. anethi* 11201. *A. alternata* 11214, *F. avenaceum* 11219, *F. sambucinum* 11226 and *F. sporotrichioides* 11222 demonstrated lower aggressiveness on the common caraway seedlings *in vitro* (Table 2). Positive correlation ($r = 0.657$) between seedling length and number of healthy plants and negative correlation ($r = -0.520$) between seedling length and number of dead or injured plants significant at 0.01 level were established.

Table 1. The occurrence of potential pathogens in common caraway seeds

Fungi	Number of isolates					FO %	RD %
	2001	2002	2003	2004	Total		
Alternaria alternata (Fr.) Keissl.	103	213	659	700	1675	31.31	32.62
A. dauci (J. G. Kühn) J. W. Groves & Skolko	0	0	0	24	24	0.45	0.47
A. petroselini (Neerg.) E. G. Simmons	0	0	11	0	11	0.21	0.21
A. radicina Meier, Drechsler & E. D. Eddy	57	105	85	89	336	6.28	6.54
Ascochyta biforae Bond.-Mont.	0	2	25	14	41	0.77	0.80
Ascochyta sp.	0	0	5	1	6	0.11	0.12
Botrytis cinerea Pers.	10	3	0	12	25	0.47	0.49
Cylindrocarpon destractans (Zinssm.) Scholten (*Neonectria radicicola* (Gerlach & L. Nilsson) Mantiri & Samuels)	0	1	0	0	1	0.02	0.02
Fusarium avenaceum (Fr.) Sacc. (*Gibberella avenacea* R. J. Cook)	0	2	30	7	39	0.73	0.76
F. oxysporum Schltdl.	0	0	13	0	13	0.24	0.25
F. solani (Mart.) Sacc. (*Haematonectria haematococca* (Berk. & Broome) Samuels & Rossman	0	1	3	0	4	0.07	0.08
Helminthosporium sp.	6	0	0	0	6	0.11	0.12
Mortierella sp.	2	0	0	0	2	0.04	0.04
Phoma anethi (Pers.) Sacc. *(Mycosphaerella anethi* (Pers.) Petr.)	0	0	0	1	1	0.02	0.02
Phoma eupyrena Sacc.	0	0	0	1	1	0.02	0.02
Phoma spp.	9	36	3	12	60	1.12	1.17
Phomopsis diachenii Sacc.	0	0	42	0	42	0.79	0.82
Phomopsis sp.	0	0	3	5	8	0.15	0.16
Rhizoctonia sp.	2	0	1	2	5	0.09	0.10
Sclerotinia sclerotiorum (Lib.) de Bary	1	0	0	0	1	0.02	0.02
Septoria umbelliferarum Kalchbr.	0	0	0	1	1	0.02	0.02
Septoria sp.	0	0	0	1	1	0.02	0.02
Stemphylium botryosum Sacc.	6	36	1	0	43	0.80	0.84
S. sarciniforme (Cavara) Wiltshire	0	1	0	0	1	0.02	0.02
Verticillium albo-atrum Reinke & Berthold	0	0	7	0	7	0.13	0.14

FO – frequency of occurrence, RD – relative density

Table 2. The pathogenicity of fungi, isolated from common caraway seeds for 'Record' seedlings *in vitro*

Fungus species, strain	Length of seedlings				Number of seedlings %		
	after 14 days		after 30 days		dead	injured	healthy
	mm	%*	mm	%			
Control	53.3 ± 5.0^{x}	100.0	133.5 ± 5.7^{x}	100.0	0^{x}	0^{x}	100.0^{x}
Alternaria alternata 11214	54.8 ± 8.2	102.8	113.6 ± 5.6	85.1	0	20.0	80.0
Fusarium avenaceum 11212	26.8 ± 5.4^{x}	50.3	–	–	100.0^{x}	0	0^{x}
F. avenaceum 11219	62.2 ± 4.2	116.7	105.6 ± 15.0	79.1	0	80.0^{x}	20.0^{x}
F. avenaceum 11220	64.4 ± 13.6	120.8	88.2 ± 20.7^{x}	66.1	40.0^{x}	60.0^{x}	0^{x}
F. sambucinum 11226	49.8 ± 7.4	93.4	114.4 ± 23.8	85.7	20.0	0	80.0
F. sporotrichioides 11222	63.6 ± 4.7	119.3	115.8 ± 4.9	86.7	0	20.0	80.0
Phoma anethi 11201	39.8 ± 4.6^{x}	74.7	50.4 ± 4.2^{x}	37.8	80.0^{x}	0	20.0^{x}

Note. %* – calculated from control, ^{x} – marked values in each column are significantly different from control at 0.05 level.

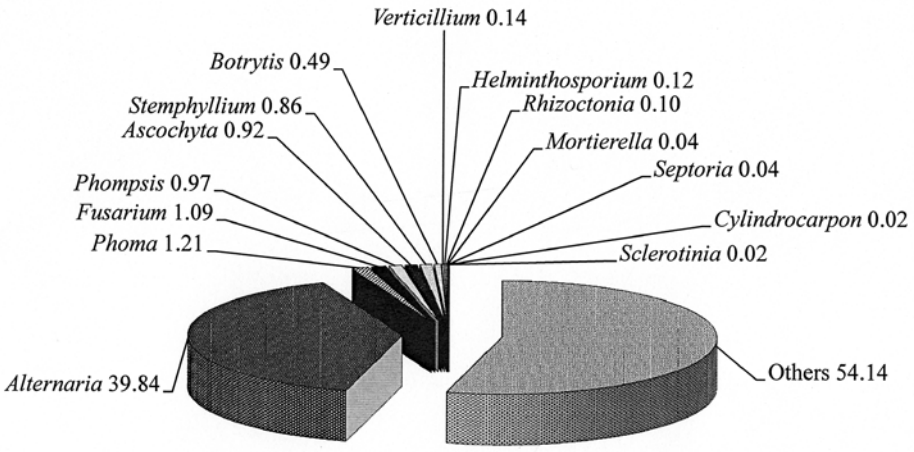

Figure 1. The occurrence of potential pathogens' genera in common caraway seeds (RD % among total isolates)

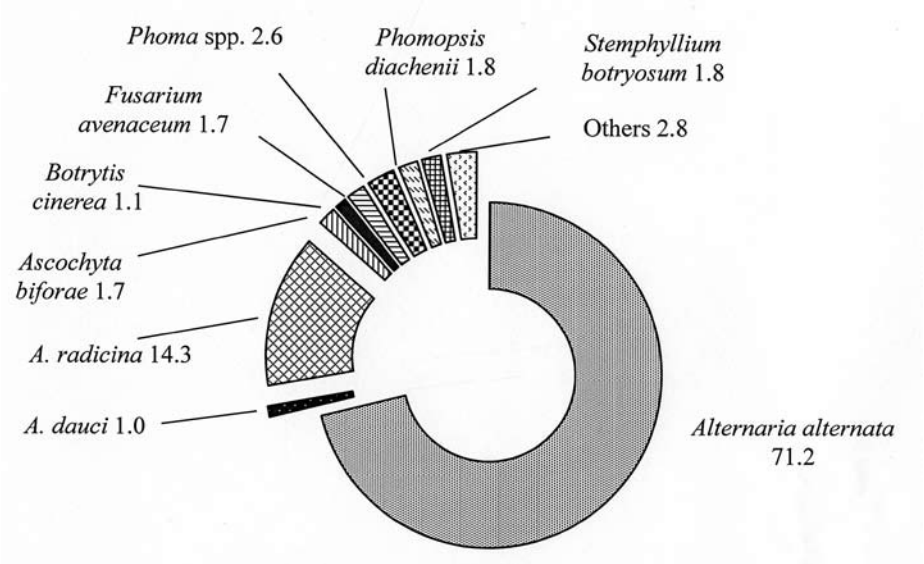

Figure 2. The occurrence of most frequent potential pathogens in common caraway seeds (RD % among pathogens)

The study of resistance of the three common caraway vatieties ('Gintaras', 'Kančevitskij', 'Rekord') to *F. avenaceum* 11212 and *P. anethi* 11201 indicated 'Kančevitskij' to be most resistant among the studied varieties, whereas 'Rekord' exhibited the lowest resistance to the tested disease agents. However, the evaluation of common caraway wintering performance showed the best over winter survival of the variety 'Rekord', while the lowest survival was exhibited by the 'Kančevitskj' plants. It should be noted that pathogen-infected plants exhibited a poor over winter survival compared to the control. The highest number of dead plants (40–75%) after winter was detected in the treatment infected with *F. avenaceum* 11212 (Table 3).

Table 3. The resistance of common caraway varieties to *Fusarium avenaceum* and *Phoma anethi*

Treatment	Healthy seedlings %					
	'Gintaras'		'Kančevitskij'		'Rekord'	
	I	II	I	II	I	II
Control (not infected)	100 ± 0^{a}	76.7 ± 0.9^{a}	100 ± 0^{a}	63.3 ± 0.9^{a}	100 ± 0^{a}	86.7 ± 0.3^{a}
Infected with *F. avenaceum* 11212	63.0 ± 0.3^{b}	36.8 ± 0.9^{b}	67.0 ± 0.3^{b}	25.0 ± 1.2^{b}	50.0 ± 0.6^{b}	60.0 ± 0.6^{b}
Infected with *P. anethi* 11201	70.0 ± 0^{c}	57.1 ± 0.6^{c}	73.0 ± 0.7^{c}	45.5 ± 1.2^{c}	60.0 ± 0.6^{c}	66.7 ± 1.2^{c}

Notes. The evaluation of seedlings injury: I – after 30 days and at the end of the first year's vegetation period, II – after overwintering and at the end of the second year's vegetation period. Means followed by the different letters within each column are significantly different at $p \leq 0.05$.

Infected common caraway plants produced seeds of lower quality. Infestation with *F. avenaceum* 11212 worsened seed quality mostly. The plants infected with this pathogen produced seeds of considerably lower germination. Seed germination and germinating power of 'Kančevitskij' decreased by 34.0% and 38.1%, and of Gintaras' by 23.4% and 20.4%, respectively compared to the control. The pathogen infection had lowest impact on *C. carvi* 'Rekord' seed quality (Table 4).

Table 4. Seed quality indices of common caraway, infected with *Fusarium avenaceum* and *Phoma anethi*

Var. No.	'Gintaras'			'Kančevitskij'			'Rekord'		
	Germination %	Germinating power %	1000 seeds weight g	Germination %	Germinating power %	1000 seed weight g	Germination %	Germinating power %	1000 seed weight g
I	80.0 ± 1.2^{a}	68.7 ± 0.7^{a}	1.74 ± 0.01^{a}	89.0 ± 0.7^{a}	84.0 ± 1.2^{a}	1.76 ± 0.02^{a}	88.0 ± 1.2^{a}	82.0 ± 1.2^{a}	2.03^{a} ±0.11
II	61.3 ± 1.3^{b}	54.7 ± 1.8^{b}	1.56 ± 0.09^{b}	58.7 ± 1.8^{b}	52.0 ± 1.2^{b}	1.71 ± 0.03^{ab}	80.7 ± 1.8^{b}	75.3 ± 1.8^{b}	2.02^{a} ± 0.09
III	66.7 ± 1.8^{c}	63.3 ± 0.7^{c}	1.68 ± 0.004^{a}	88.7 ± 0.7^{ba}	82.7 ± 1.8^{ba}	1.60 ± 0.09^{b}	85.3 ± 1.8^{ab}	78.0 ± 1.2^{bc}	1.94^{a} ± 0.16

Notes. I – control (not infected); II– infected with F. avenaceum 11212; III – infected with P. anethi 11201. Means followed by the same letters within each column are not significantly different at $p \leq 0.05$.

In search of measures reducing the impact of potential pathogens on the common caraway, the effect of six fungicides (Dithane M-45, Efector, Euparen Multi 500 WG, Previcur 607 SL, Signum 334 WG, Zato 50 WG) and three seed treatments (Kemikar T, Cruiser OSR, Maxim 025 FS) on the mycelium growth *in vitro* of eight fungal strains, most frequent in common caraway seed, were tested. The following strains were investigated: *A. alternata* 11215, 11331, *A. radicina* 11332, *F. avenaceum* 11212, 11335, *F. sporotrichioides* 11337, *P. anethi* 11201, *U. oudemansii* 11334. Studies have shown that the tested agents inhibited the growth of fungi *in vitro*. Among the fungicides Signum was the most effective, significantly decreased the growth of all the tested fungi and of some of them even stopped (*P. anethi* 11201, *U. oudemansii* 11334, *F. avenaceum* 11335). Zato demonstrated slightly weaker effect, especially against *Fusarium* fungi. The mycelium of investigated fungi surrounded the disks moistened with this fungicide, but did not overgrow them. Dithane stopped *P. anethi* 11201 and *A. alternata* 11215 growth after 10 days only. Other fungicides, while slowing down the growth of mycelium, did not stop it and the mycelium gradually overgrew the discs. Previkur had the weakest effect on growth of the tested fungi. This fungicide slightly stopped growth *U. oudemansii* 11334 and *A. alternata* 11215 only, but on the 10th day of growth, most of tested fungi filled Petri dishes completely and luxuriantly overgrew disks moistened with this preparation (Table 5).

Table 5. The impact of fungicides, seed treatments and caraway oil on fungi growth *in vitro*

Treatment	CD mm	CGI %	CD mm	CGI %	CD mm	CGI %
	after 3 days		after 10 days		after 20 days	
1	2	3	4	5	6	7
			Phoma anethi 11201			
Control	60.8 ± 0.8	0	90.0 ± 0	0	–	–
Euparen	30.7 ± 1.9	49.6 ± 1.4	47.2 ± 3.3	44.6 ± 0.8	52.5 ± 0.6	–
Efector	22.3 ± 0.3	63.2 ± 0.7	50.7 ± 2.2	43.7 ± 1.8	90.0 ± 0	–
Signum	16.3 ± 0.3	73.1 ± 0.7	17.7 ± 0.7	80.4 ± 0.7	17.7 ± 0.7	–
Zato	30.3 ± 1.8	50.1 ± 2.9	47.4 ± 1.7	47.2 ± 1.9	52.2 ± 0.3	–
Dithane	40.8 ± 0.9	32.8 ± 1.6	55.5 ± 0.8	38.3 ± 0.9	55.5 ± 0.8	–
Previcur	22.3 ± 0.9	63.3 ± 1.5	68.8 ± 0.7	23.5 ± 0.8	90.0 ± 0	–
Maxim	22.2 ± 1.0	63.5 ± 1.7	28.0 ± 0.6	68.9 ± 0.6	44.7 ± 1.2	–
Cruiser	21.5 ± 0	64.6 ± 0	27.8 ± 0.7	69.1 ± 0.8	29.2 ± 0.8	–
Kemikar	24.5 ± 0.3	59.7 ± 0.5	41.2 ± 1.0	52.4 ± 1.1	51.5 ± 0.3	–
Caraway oil	18.8 ± 0.4	69.0 ± 0.7	18.8 ± 0.4	79.1 ± 0.5	18.8 ± 0.4	–
			Alternaria alternata 11215			
Control	61.2 ± 0.7	0	90.0 ± 0^{x}	0	–	–
Euparen	43.3 ± 0.7	29.2 ± 1.1	90.0 ± 0^{x}	0	–	–
Efector	43.3 ± 1.1	29.2 ± 1.8	90.0 ± 0^{x}	0	–	–
Signum	25.2 ±0.8	58.9 ± 1.4	28.7 ± 0.4	68.1 ± 0.5	43.2 ± 0.6	–
Zato	35.3 ± 0.7	42.3 ± 1.1	53.3 ± 0.7	40.7 ± 0.7	58.0 ± 0.6	–
Dithane	36.0 ± 0.9	41.2 ± 1.4	53.5 ± 0.3	40.6 ± 0.3	53.5 ± 0.3	–
Previcur	55.3 ± 0.9	9.6 ± 1.4	90.0 ± 0^{x}	0	–	–
Maxim	27.3 ± 0.2	55.4 ± 0.3	36.8 ± 0.9	59.1 ± 1.0	53.3 ± 0.2	–
Cruiser	26.8 ± 1.2	56.2 ± 2.0	26.8 ± 1.2	70.2 ± 1.4	38.8 ± 2.9	–
Kemikar	22.5 ± 0.3	63.2 ± 0.5	29.8 ± 1.2	66.8 ± 1.3	46.2 ± 0.6	–
Caraway oil	–	–	–	–	–	–

Table 5 continued

1	2	3	4	5	6	7
			Alternaria alternata 11331			
Control	49.5 ± 0.5	0	90.0 ± 0	0	–	–
Euparen	32.2 ± 0.5	34.4 ± 1.0	58.3 ± 1.7	35.2 ± 1.9	90.0 ± 0	–
Efector	32.7 ± 0.6	34.0 ± 1.2	72.2 ± 0.3	19.8 ± 0.4	–	–
Signum	26.3 ± 0.6	46.8 ± 1.2	34.2 ± 1.7	62.0 ± 1.9	56.0 ± 0.9	–
Zato	28.5 ± 0.3	42.4 ± 0.6	43.3 ± 1.3	51.8 ± 1.5	73.7 ± 1.8	–
Dithane	33.0 ± 0.3	33.3 ± 0.6	73.8 ± 0.9	18.0 ± 1.0	90.0 ± 0	–
Previcur	24.8 ± 0.7	49.8 ± 1.3	40.3 ± 0.2	55.2 ± 0.2	60.8 ± 1.4	–
Maxim	32.2 ± 0.7	35.0 ± 1.5	55.0 ± 0.8	38.9 ± 0.9	57.6 ± 0.6	–
Cruiser	17.0 ± 0.8	65.6 ± 1.6	30.0 ± 0.8	66.7 ± 0.8	58.2 ± 2.4	–
Kemikar	26.7 ± 0.9	46.1 ± 1.9	29.8 ± 1.1	66.8 ± 1.2	54.1 ± 1.9	–
Caraway oil	20.0 ± 0.3	59.6 ± 0.6	20.0 ± 0.3	77.8 ± 0.3	90.0 ± 0	–
			Alternaria radicina 11332			
Control	39.2 ± 0.4	0	90.0 ± 0	0	–	–
Euparen	29.7 ± 1.1	24.3 ± 2.8	48.7 ± 0.4	46.6 ± 1.1	90.0 ± 0	–
Efector	27.3 ± 0.7	30.3 ± 1.7	42.3 ± 0.9	52.9 ± 1.0	75.8 ± 2.1	–
Signum	19.2 ± 0.3	51.1 ± 0.8	21.8 ± 1.6	75.7 ± 1.8	29.0 ± 0.5	–
Zato	16.8 ± 0.4	57.1 ± 1.1	33.3 ± 1.9	63.0 ± 2.1	61.5 ± 1.4	–
Dithane	27.8 ± 0.2	29.0 ± 0.4	46.5 ± 1.0	48.3 ± 1.2	60.7 ± 0.9	–
Previcur	36.0 ± 0.6	8.2 ± 1.5	53.5 ± 0.3	40.6 ± 0.3	72.8 ± 0.4	–
Maxim	19.0 ± 0.3	51.5 ± 0.7	19.0 ± 0.3	78.9 ± 0.3	43.3 ± 1.3	–
Cruiser	28.7 ± 0.9	26.9 ± 12.4	51.2 ± 0.9	43.1 ± 1.0	90.0 ± 0	–
Kemikar	20.0 ± 0.3	49.0 ± 0.8	27.3 ± 0.4	69.6 ± 0.5	90.0 ± 0	–
Caraway oil	19.2 ± 0.6	51.1 ± 1.5	19.2 ± 0.6	78.7 ± 0.7	90.0 ± 0	–
			Ulocladiun oudemansii 11334			
Control	45.5 ± 0.3	0	90.0 ± 0[x]	0[x]	–	–
Euparen	31.0 ± 0.5	31.2 ± 1.1	90.0 ± 0[x]	0[x]	–	–
Efector	28.2 ± 0.2	38.1 ± 0.4	70.7 ± 0.3	21.5 ± 0.4	–	–
Signum	16.7 ± 0.3	63.3 ± 1.0	16.7 ± 0.3	81.5 ± 0.4	16.7 ± 0.3	–
Zato	23.8 ± 0.2	39.2 ± 1.0	90.0 ± 0[x]	0[x]	–	–
Dithane	27.7 ± 0.4	39.2 ± 1.0	56.7 ± 0.9	37.0 ± 1.0	90.0 ± 0	–
Previcur	43.3 ± 0.4	4.8 ± 1.0	90.0 ± 0[x]	0[x]	–	–
Maxim	17.2 ± 0.8	62.2 ± 1.8	19.3 ± 0.7	78.3 ± 0.8	43.5 ± 0.8	–
Cruiser	16.0 ± 0.8	64.7 ± 1.7	18.5 ± 0.3	79.8 ± 0.2	90.0 ± 0	–
Kemikar	19.2 ± 0.4	58.0 ± 0.8	32.7 ± 1.5	63.7 ± 1.6	51.7 ± 0.3	–
Caraway oil	16.2 ± 0.2	64.4 ± 0.4	16.2 ± 0.2	82.0 ± 0.2	16.2 ± 0.2	–
			Fusarium avenaceum 11212			
Control	69.8 ± 0.4	0	90.0 ± 0[x]	0[x]	–	–
Euparen	48.0 ± 0.5	31.2 ± 0.7	90.0 ± 0[x]	0[x]	–	–
Efector	45.3 ± 1.1	35.0 ± 1.6	90.0 ± 0[x]	0[x]	–	–
Signum	30.3 ± 0.3	56.5 ± 0.5	48.5 ± 0.9	46.1 ± 1.0	57.2 ± 1.6	–
Zato	42.2 ± 0.6	39.6 ± 0.9	72.5 ± 1.3	19.5 ± 1.4	90.0 ± 0	–
Dithane	47.8 ± 0.7	31.5 ± 1.0	90.0 ± 0[x]	0[x]	–	–
Previcur	67.3 ± 0.2	3.5 ± 0.2	90.0 ± 0[x]	0[x]	–	–
Maxim	33.3 ± 1.1	52.3 ± 1.6	43.3 ± 3.6	52.0 ± 4.0	48.3 ± 2.7	–
Cruiser	25.0 ± 1.5	64.2 ± 2.2	26.2 ± 1.4	70.9 ± 1.5	33.0 ± 1.3	–
Kemikar	29.5 ± 0.8	57.7 ± 1.1	34.5 ± 1.0	61.7 ± 1.2	41.0 ± 1.0	–
Caraway oil	25.3 ± 0.9	63.7 ± 1.3	25.3 ± 0.9	71.8 ± 1.0	90.0 ± 0	–
			Fusarium avenaceum 11335			
Control	73.2 ± 0.2	0	90.0 ± 0[x]	0[x]	–	–
Euparen	68.2 ± 0.6	6.8 ± 0.8	90.0 ± 0[x]	0[x]	–	–
Efector	45.5 ± 1.0	37.9 ± 1.4	90.0 ± 0[x]	0[x]	–	–
Signum	27.3 ± 0.3	62.6 ± 0.5	42.5 ± 1.0	52.8 ± 1.1	42.5 ± 1.0	–
Zato	47.5 ± 0.8	35.1 ± 1.0	77.3 ± 0.2	14.1 ± 0.2	90.0 ± 0	–
Dithane	50.5 ± 1.3	31.0 ± 1.7	90.0 ± 0[x]	0[x]	–	–
Previcur	62.8 ± 1.3	14.2 ± 1.8	90.0 ± 0[x]	0[x]	–	–
Maxim	36.5 ± 1.8	50.1 ± 2.5	45.8 ± 0.7	49.1 ± 0.8	55.5 ± 1.0	–
Cruiser	37.5 ± 2.8	48.8 ± 3.9	52.2 ± 0.7	42.0 ± 0.8	54.8 ± 0.7	–
Kemikar	31.3 ± 0.3	57.2 ± 0.5	39.7 ± 0.9	55.9 ± 1.0	43.0 ± 0.3	–
Caraway oil	–	–	–	–	–	–

Table 5 continued

1	2	3	4	5	6	7
			Fusarium sporotrichioides 11337			
Control	90.0 ± 0[x]	0[x]	–	–	–	–
Euparen	90.0 ± 0[x]	0[x]	–	–	–	–
Efector	90.0 ± 0[x]	0[x]	–	–	–	–
Signum	73.0 ± 0.1	18.9 ± 0.6	90.0 ± 0[x]	–	–	–
Zato	71.7 ± 0.2	20.3 ± 1.9	90.0 ± 0[x]	–	–	–
Dithane	70.5 ± 0.2	21.7 ± 2.6	90.0 ± 0[x]	–	–	–
Previcur	90.0 ± 0[x]	0[x]	90.0 ± 0[x]	–	–	–
Maxim	70.7 ± 0.03	21.5 ± 0.4	90.0 ± 0[x]	–	–	–
Cruiser	70.0 ± 0.02	22.2 ± 0.3	90.0 ± 0[x]	–	–	–
Kemikar	69.5 ± 0.1	22.8 ± 1.7	90.0 ± 0[x]	–	–	–
Caraway oil	42.0 ± 0.1	53.3 ± 0.9	90.0 ± 0[x]	–	–	–

Notes. CD – colony diameter, mm, CGI – colony growth inhibition, %. All mean differences from control, except marked ([x]) ones, in each column are significant at 0.05 level. The values of CD after 20 days were not statistically evaluated.

The impact of seed treatments on the fungal growth was similar. They all inhibited the mycelium growth of the tested fungi. Fungal mycelium at the place of its contiguity with discs sustained lyses and did not overgrow the discs. However, the growth did not stop and continued between discs after 10 days. Cruiser demonstrated the highest inhibitory effect on most of the tested fungi, especially on *P. anethi* 11201, *A. alternata* 11215 and *F. avenaceum* 11212. Maxim was most effective against *A. radicina* 11332 and *U. oudemansii* 11334 mycelium growth, whereas Kemicar – against *F. avenaceum* 11335 (Table 5). These preparations had the least impact on the mycelium growth of *F. sporotrichioides* 11337, which luxuriantly overgrew discs moistened with them. This fungus was most resistant to the impact of all fungicides tested as well. Although many tested preparations slightly stopped the mycelium growth of this fungus, it continued to grow, luxuriantly overgrew disks and after 10 days completely filled Petri dishes (Table 5).

The effect of caraway essential oil on the mycelium growth of six fungi isolated from *C. carvi* seeds (*Alternaria alternata* 11331, *A. radicina* 11332, *Fusarium avenaceum* 11212, *F. sporotrichioides* 11337, *Phoma anethi* 11201, and *Ulocladium oudemansii* 11334) was very conspicuous during the first growing days. The influence of essential oil considerably slowed down the growth of fungal mycelium and its luxuriance. Colony growth inhibition of most of the tested fungi amounted up to 51.1–69.0% on the third day of growth. Their mycelium did not grow till the 10th day. However, the impact of essential oil on the growth of many of the tested fungi disappeared after 20 days and their mycelium luxuriantly overgrew the discs. The growth stopped only for *P. anethi* 11201 and *U. oudemansii* 11334 mycelium (Table 5). The study results suggest that the content of essential oil in common caraway seeds has an impact on the spread of fungi in them and imply that seeds accumulating more essential oils should be less contaminated with fungi. Weakening effect of the essential oil on fungi growth over time, might be explained by its decreasing concentration in the environment.

It is known that some bacteria inhibit the growth of fungi and are successfully used as a fungal antagonist, in order to reduce the harmfulness of fungi (Moline et al., 1999; Wang et al., 2003). We tested the impact of five saprotrophic *Erwinia* strains, isolated from common caraway seeds, on the mycelium growth of *Alternaria alternata* 11215, *Fusarium avenaceum* 11212, *F. culmorum* 11211, *F. heterosporum* 11210, and *Phoma anethi* 11201 *in vitro*. The investigation evidenced that at first the bacteria inhibit growth of fungus, but on the 10th day of the common growth the mycelium of most of the tested fungi comes near to the bacteria colonies and starts to overgrow them. Strain No. 1136 exhibited the highest antagonism. Due to its impact, the mycelium growth of all the tested fungi slowed down markedly and it was not as exuberant as in the control treatment. The strains No. 1128 and No. 1134 stopped growth of *Alternaria alternata* 11215 and *Phoma anethi* 11201 mycelium (Fig. 3).

Erwinia 1128 × *Alternaria alternata* 11215

Erwinia 1136 × *Phoma anethi* 11201

Erwinia 1136 × *Fusarium avenaceum* 11212

Figure 3. The impact of *Erwinia* genus bacteria on fungi growth *in vitro* after 25 days (left – control)

Our research evidenced that the spread of potential pathogens in common caraway seeds depends on harvest year and cultivation locality. The pathogenic strains which can be responsible for diseases incidence in crops were ascertained among them. In our experiment, they caused decay of common caraway seedlings and their aggressiveness depended on the species and strain of pathogen. The study showed poor overwintering of infected plants, which resulted in seed quality deterioration. Consequently the losses in yield and its quality can be sustained unless appropriate control measures are introduced. Fungicide application and the use of resistant varieties are the main means of disease control in disease management of many crops. Unfortunately, the lack of approved fungicides and their toxicity limit the use of chemical agents in medicinal and spice crops. Chemical disease management has proved impractical, mainly due to the appearance of fungicide-resistant strains, concern over the presence of chemical residues in the food chain, and problems of environmental pollution (Chérif et al., 2002). Therefore, despite the fact that the tested fungicides had fungistatic effect on pathogens in our investigation, their application is problematic. In the development of environmentally friendly control strategies biological control has emerged as a harmless alternative. The use of microbial agents has shown significant potential. Therefore the search for potential application of antagonistic bacteria in the control of caraway seed pathogens as one of the biocontrol measures might be advisable. The spread of pathogens can be reduced by using resistant varieties which accumulate higher contents of essential oils.

Conclusions

1. The fungi of 18 species and 14 genera attributable to potential pathogens, the agents of plant spots, wilts and rots, were identified in common caraway seeds. They were detected in almost all seed samples annually and their frequency of occurrence amounted to up to 44.0% in total.

2. The fungi of *Alternaria* genus prevailed and their isolates accounted for 86.9% of the total amount of potential pathogens. *Phoma* spp., *Phomopsis diachenii*, *Stemphylium botryosum*, *Ascochyta biforae*, *Fusarium avenaceum*, *Botrytis cinerea* were more frequent among potential pathogens detected. Although the incidence of some of the potential pathogens was not very high, in the individual seed samples it was quite large and could be important in the spread of diseases in caraway crops.

3. The potential pathogens in infected seeds of cultivated and wild caraway had very high qualitative and quantitative similarity (Sorenson's indices (SI) 68.4% and 92.2%, accordingly), however they significantly differed between years and localities.

4. Aggressive strains (*Fusarium avenaceum* 11212 and *Phoma anethi* 11201), injuring caraway seedlings *in vitro*, were ascertained among the potential pathogens tested. *Carum carvi* 'Kančevitskij' was most resistant to them. In pot trials, infected plants exhibited a poorer over-winter survival and produced seeds of lower quality.

5. The tested fungicides, common caraway essential oil as well as tested strains of *Erwinia* genus bacteria produced fungistatic effect on seed pathogens of common caraway.

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Paprastojo kmyno (*Carum carvi* L.) sėklų potencialūs patogenai ir jų plitimą stabdančios priemonės

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Santrauka

Tirtas laukinio ir kultūrinio paprastojo kmyno (*Carum carvi* L.) 2001–2004 m. derliaus sėklų užsikrėtimas potencialiais patogenais. Sėklos surinktos joms subrendus, birželio–liepos mėnesiais, įvairiose augavietėse Biržų, Kauno, Raseinių, Šilutės, Ukmergės, Utenos, Varėnos ir Vilniaus rajonuose.

Paprastojo kmyno sėklose buvo aptikti ir identifikuoti 18 rūšių bei 14 genčių grybai, priskirtini potencialiems patogenams, augalų dėmėtligių, vytulių ir puvinių sukėlėjams. Jie buvo aptikti beveik visuose sėklų ėminiuose kiekvienais metais, o jų aptikimo dažnis siekė 44,0 %. Tirtose sėklose vyravo *Alternaria* genties grybai, sudarę 86,9 % bendro potencialių patogenų izoliatų kiekio. Dažniau buvo aptinkami ir *Phoma* spp., *Phomopsis diachenii*, *Stemphylium botryosum*, *Ascochyta biforae*, *Fusarium avenaceum*, *Botrytis cinerea*. Nors kai kurių potencialių patogenų aptikimo dažnis nėra labai didelis, kai kuriuose sėklų ėminiuose jie aptinkami gana gausiai ir gali būti reikšmingi ligų plitimui paprastojo kmyno pasėliuose. Laukinių ir kultūrinių kmynų sėklose nustatyti potencialūs patogenai turi labai didelį kokybinį bei kiekybinį panašumą, tačiau jie gerokai skiriasi įvairių augaviečių ir skirtingų derliaus metų kmynų sėklose.

Tiriant paprastojo kmyno sėklose aptiktų grybų patogeniškumą, nustatyti agresyvūs *Fusarium avenaceum* 11212 ir *Phoma anethi* 11201 kamienai. Jie pažeidė kmynų daigus *in vitro*. Šiems patogenams buvo atspariausias veislės ‘Kančevitskij’ paprastasis kmynas. Vegetacinio bandymo rezultatai parodė, kad infekuoti augalai sunkiau peržiemoja ir subrandina prastesnės kokybės sėklas.

Kadangi cheminių priemonių taikymas paprastojo kmyno pasėliuose yra problemiškas, atsparių veislių, sukaupiančių daugiau eterinių aliejų ir antagonistinių bakterijų, panaudojimas galėtų būti reikšminga alternatyva kontroliuojant sėklų patogenus.

Reikšminiai žodžiai: *Carum carvi*, sėklos, grybai, patogenai, aptikimo dažnis, patogeniškumas, atsparumas, kontrolė.