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Internal mycobiota of wild and cultivated common caraway (*Carum carvi* L.) seeds

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Abstract

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

The fungi of 55 species, two varieties and 39 genera, belonging to *Ascomycota*, *Basidiomycota* and *Zygomycota* phyla, *Sordariomycetes*, *Dothideomycetes*, *Leotiomycetes*, *Eurotiomycetes*, *Sacharomycetes*, *Agaricomycetes* and *Incertae sedis* classes were detected and identified inside of common caraway seeds. The micromycetes of genus *Alternaria* dominated in the internal mycobiota of investigated seeds. They made up 48.1% of the total isolate amount and their frequency of occurrence amounted to 47.1%. *Alternaria alternata*, *Ascochyta biforae*, *Botrytis cinerea*, *Fusarium* spp., *Phoma* spp., *Phomopsis* spp., *Rhizoctonia* sp., *Verticillium albo-atrum* – the agents of plant spots, wilts and rots were observed inside the common caraway seeds, although the saprotrophes (*Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Ulocladium* spp. and others), potential producers of mycotoxins, prevailed. The fungal complexes, detected inside of wild and cultural caraway seeds, has very great qualitative and great quantitative similarity, however they significantly vary between harvest years and localities. The greater similarity was ascertained among fungal complexes, identified inside seeds of cultural caraway, compare with fungal complexes in wild caraway seeds.

Key words: *Carum carvi*, fungi, seeds, frequency of occurrence, relative density.

Introduction

In recent years, spices and herbal medicines, including caraway, have become increasingly common in our daily diet and play an important role in the economy (Romagnoli et al., 2007). Over the last few decades the growing and processing of medicinal raw material have become a business that has increased significantly in Europe and all over the world (Ragažinskienė, 2004). Growing medicinal and spice plants, their exploitation in pharmaceutical, food, cosmetics and other processing industries belong to the most significant developing fields in world. Seed quality (genetic purity, viability and vigour, moisture content, size and appearance, mechanical damage) and health is an important factor for the successful cultivation of crops, including caraway. Seed health refers to the presence or absence of disease-causing organisms, such as fungi, bacteria and viruses, and animal pests (Icishahayo et al., 2009). Fungi form a major group of pathogens that are seed-borne as well as seed-transmitted (Singh, Mathur, 2004). Many fungi are serious parasites of seed primordia, maturing and stored seeds and their invasion can result in various damage including reduced quantitative and qualitative seed yield, discolorations, mycotoxins contamination, decreased germination and total decay (Embaby, Abdel-Galil, 2006). They occur with seed either as contaminants adhering to the seed surface, loosely mixed with seed, or as an infection present inside the seed tissues (Singh, Mathur, 2004). Internal seed infection is particularly important. In many cases the presence of fungi within seed is asymptomatic and cannot be evaluated by visual observation, nevertheless they cause a more severe seed damage and contamination by mycotoxins, and may be transmitted to other crops.

Despite many studies on the mycobiota and mycotoxins in seeds and grain of agricultural crops, only a few deal with spice and medicinal herbs and especially with caraway seeds. In Lithuania, the mycobiota of common caraway seeds has not yet been investigated.

The present study was conducted to ascertain the extent of internal fungal infection of common caraway seeds, to identify the species diversity of micromycetes occurring inside the seeds, to evaluate their distribution and to compare the fungal complexes within the cultural and wild common caraway seeds as well as in the seeds of different harvest years and various localities.

Materials and methods

The internal fungal infection of cultivated ('Gintaras', 'Kančevitskij', 'Rekord') and wild common caraway (*Carum carvi* L.) seeds of 2001–2004 harvest was studied. 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 pure culture method was applied for the detection of micromycetes inside the seeds (Mathur, Kongsdal, 2003). The seeds were sterilized with 75% ethanol for 1 min, rinsed with sterile water for three times and drained with sterile filter paper. The surface sterile seeds were plated in Petri dishes (10 seeds per Petri dish) on malt extract agar (MEA) and synthetic nutrient agar (SNA) (Singleton et al., 1993), with streptomycin (250 mg l^{-1}) addition and incubated for seven days at 24°C in the dark. The analysis of each seed sample was performed in two replications (100 seeds per replication). After incubation, the fungi developed from the internal tissues of each seed were estimated. They were identified based on their morphological and cultural characteristics according to Ellis (1971; 1976), Nelson et al. (1983), Arx et al. (1986), Melnik (2000). The frequency of occurrence (FO) of individual 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 tested common caraway seeds were calculated (González et al., 1995). The FO indicates the distribution of particular species in tested object as the RD – its abundance against other fungus species detected there. The SPSS 17 statistical package was used to analyze the obtained data. The qualitative and quantitative similarity of fungal complexes, ascertained in the seeds of cultural 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 NRC, Institute of Botany Vilnius, Lithuania.

Results and discussion

Fungi of 55 species, 2 two varieties and 39 genera, belonging to *Ascomycota*, *Basidiomycota* and *Zygomycota* phyla, *Sordariomycetes*, *Dothideomycetes*, *Leotiomycetes*, *Eurotiomycetes*, *Saccharomycetes*, *Agaricomycetes* and *Incertae sedis* classes were detected and identified inside of investigated common caraway seeds (Table 1).

Table 1. Fungi inside of common caraway seeds

Fungi	Number of isolates					FO	RD
	2001	2002	2003	2004	Total	%	%
1	2	3	4	5	6	7	8
Acremoniella atra (Corda) Sacc.	0	3	14	0	17	0.5	0.5
Acremonium implicatum (J. C. Gilman & E. V. Abbott) W. Gams	0	0	1	0	1	0.03	0.03
A. strictum W. Gams	2	0	3	0	5	0.2	0.2
Alternaria alternata (Fr.) Keissl.	38	152	417	406	1013	30.9	31.6
A. dauci (J. G. Kühn) J. W. Groves & Skolko	0	0	0	9	9	0.3	0.3
A. petroselini (Neerg.) E. G. Simmons	0	0	11	0	11	0.3	0.3
A. radicina Meier, Drechsler & E. D. Eddy	34	87	78	51	250	7.6	7.8
A. tenuissima (Kunze) Wiltshire	14	98	93	55	260	7.9	8.1
Arthrinium phaeospermum (Corda) M. B. Ellis	3	2	32	8	45	1.4	1.4
Ascochyta biforae Bond.-Mont.	0	2	25	8	35	1.1	1.1

Table 1 continued

1	2	3	4	5	6	7	8
Ascochyta sp.	0	0	5	1	6	0.2	0.2
Aspergillus spp.	18	0	412	30	460	14.1	14.4
Aureobasidium pullulans (de Bary) G. Arnaud	0	0	0	18	18	0.6	0.6
Botrytis cinerea Pers.	0	0	0	6	6	0.2	0.2
Chaetomium bostrychodes Zopf	15	0	0	0	15	0.5	0.5
C. cochliodes Palliser	0	0	0	2	2	0.06	0.06
C. globosum Kunze	0	0	18	0	18	0.6	0.6
Chrysonilia sitophila (Mont.) Arx	0	0	0	2	2	0.06	0.06
Cladosporium cladosporioides (Fresen.) G. A. de Vries	0	0	8	28	36	1.1	1.1
C. epiphyllum (Pers.) Nees	0	0	1	0	1	0.03	0.03
C. gossypiicola Pidopl. & Deniak	4	1	0	0	5	0.2	0.2
C. herbarum (Pers.) Link	5	1	32	9	47	1.4	1.5
Cylindrocarpon didymum (Harting) Wollenw.	0	0	1	0	1	0.03	0.03
Fusarium acuminatum Ellis & Everh. (*Gibberella acuminata* Wollenw.)	0	0	4	0	4	0.1	0.1
F. avenaceum (Fr.) Sacc. (*Gibberella avenacea* R. J. Cook)	0	2	23	6	31	0.9	1.0
F. graminum Corda	0	0	7	1	8	0.2	0.3
F. heterosporum Nees & T. Nees (*Gibberella gordonii* C. Booth)	0	0	1	1	2	0.06	0.06
F. incarnatum (Desm.) Sacc.	0	3	9	4	16	0.5	0.5
F. oxysporum Schltdl.	0	0	12	0	12	0.4	0.4
F. sambucinum Fuckel (*Gibberella pulicaris* (Fr.) Sacc.)	0	0	1	0	1	0.03	0.03
F. sambucinum Fuckel var. *sambucinum*	1	0	0	0	1	0.03	0.03
F. solani (Mart.) Sacc. (*Haematonectria haematococca* (Berk. & Broome) Samuels & Rossman	0	1	3	0	4	0.1	0.1
F. solani var. *argillaceum* (Fr.) Bilai (*Nectria ventricosa* C. Booth)	0	0	0	2	2	0.06	0.06
F. sporotrichioides Sherb.	0	5	0	1	6	0.2	0.2
Fusarium spp.	0	0	0	4	4	0.1	0.1
Geotrichum candidum Link	0	0	4	0	4	0.1	0.1
Leptosphaeria libanotis (Fuckel) Niessl	0	0	0	4	4	0.1	0.1
Mycelia sterilia	12	13	89	66	180	5.5	5.6
Monilia sp.	1	0	0	0	1	0.03	0.03
Mortierella sp.	2	0	0	0	2	0.06	0.06
Mucor spp.	2	7	37	11	57	1.7	1.8
Neofusicoccum mangiferae (Syd. & P. Syd.) Crous, Slippers & A. J. L. Phillips	0	8	3	1	12	0.4	0.4
Ochrocladosporium elatum (Harz) Crous & U. Braun	0	0	0	73	73	2.2	2.3
Penicillium spp.	54	0	111	14	179	5.5	5.6
Phoma anethi (Pers.) Sacc. (*Mycosphaerella anethi* (Pers.) Petr.)	0	0	0	1	1	0.03	0.03
Phoma sp.	9	12	1	4	26	0.8	0.8
Phomopsis diachenii Sacc.	0	0	12	0	12	0.4	0.4
Phomopsis sp.	0	0	3	4	7	0.2	0.2
Rhizoctonia sp.	2	0	1	2	5	0.2	0.2
Sclerotinia sclerotiorum (Lib.) de Bary	1	0	0	0	1	0.03	0.03
Scopulariopsis brevicaulis (Sacc.) Bainier (*Microascus brevicaulis* E. V. Abbott)	3	0	0	0	3	0.1	0.1
Scopulariopsis fusca Zach	1	0	0	4	5	0.2	0.2
Septoria umbelliferarum Kalchbr.	0	0	0	1	1	0.03	0.03
Septoria sp.	0	0	0	1	1	0.03	0.03

Table 1 continued

1	2	3	4	5	6	7	8
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not	0	8	40	7	55	1.7	1.7
Spilodochium vernoniae Syd.	0	0	0	2	2	0.06	0.06
Sporormiella sp.	0	0	1	0	1	0.03	0.03
Sporotrichum aurantiacum (Bull.) Fr.	0	8	14	2	24	0.7	0.8
Stemphyllium botryosum Sacc.	2	31	1	0	34	1.0	1.1
S. sarciniforme (Cavara) Wiltshire	0	1	0	0	1	0.03	0.03
Torula herbarum (Pers.) Link	0	0	0	3	3	0.1	0.1
T. ellisii Yadav & Lal	0	0	0	4	4	0.1	0.1
Trichoderma viride Pers.	0	1	13	0	14	0.4	0.4
Trichothecium roseum (Pers.) Link	0	3	3	0	6	0.2	0.2
Truncatella truncata (Lév.) Steyaert	0	0	1	0	1	0.03	0.03
Ulocladium atrum Preuss	0	0	1	1	2	0.06	0.06
U. consortiale (Thüm) E. G. Simmons	0	0	17	1	18	0.6	0.6
U. oudemansii E. G. Simmons	0	0	0	96	96	2.9	3.0
Verticillium albo-atrum Reinke & Berthold	0	0	7	0	7	0.2	0.2
Volucrispora graminea Ingold, P. J. McDougall & Dann	0	2	0	7	9	0.3	0.3

FO – frequency of occurrence %, RD – relative density %

Micromycetes from genus *Alternaria* prevailed among them and accounted for 48.1% of the total isolate amount. The relative density of *Aspergillus* spp., *Penicillium* spp. and *Ulocladium* spp. amounted to up to 14.3%, 5.6% and 3.6%, accordingly. The RD of fungi of *Fusarium*, *Cladosporium* and *Ochracladosporium* genera topped 2%, when the RD of fungi of *Mucor*, *Sordaria*, *Arthrinium*, *Ascochyta* and *Chaetomium* genera – 1% (Fig. 1). These fungi were most common in the internal tissues of common caraway seeds and their isolates made up 86.8% of the total amount of isolates.

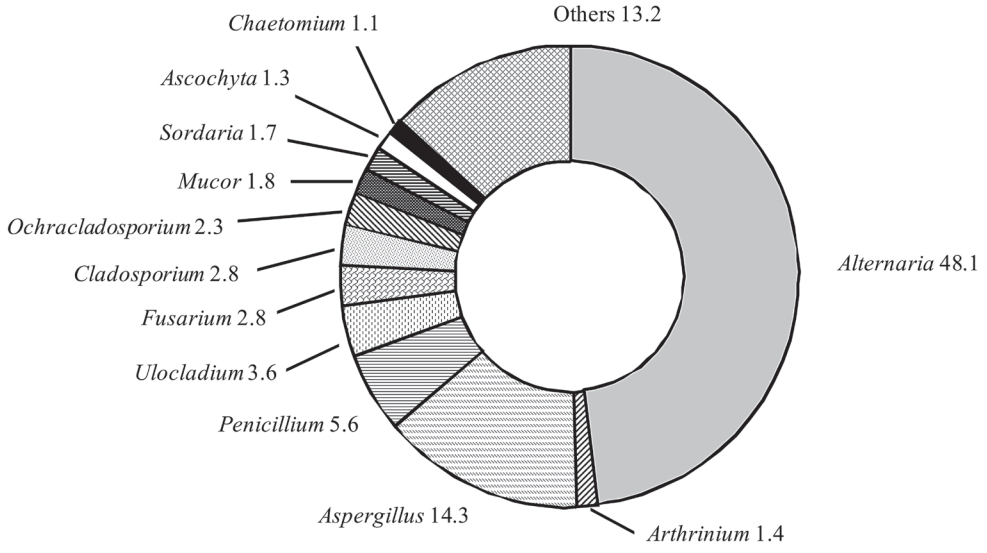

Figure 1. The relative density (%) of some fungal genera inside the common caraway seeds

The fungi of genus *Alternaria* were detected in more than four-fifths of all investigated seed samples. Their FO exceeded 47.1% and depended both on the common caraway growing locality and the harvest year. Predominantly they were found in 2002 (Fig. 3) when several *Alternaria* species were isolated from the same seed. The fungi of genus *Alternaria* were more common in the internal mycobiota of cultural caraway seeds. Their FO amounted to up to 71.5% inside seeds of cultural and up to 33.3% of wild common caraway. Five species (*A. alternata*, *A. tenuissima*, *A. radicina*, *A. dauci* and *A. petroselini*) of this genus were identified in common caraway seeds. *A. alternata* predominated

among them (FO 30.9%, RD 65.7% among *Alternaria* isolates). *A. tenuissima* and *A. radicina* were found lees frequently. Their FO amounted to 7.9% and 7.6%, RD among *Alternaria* isolates 16.9% and 16.2%, accordingly (Table 1, Fig. 2 a).

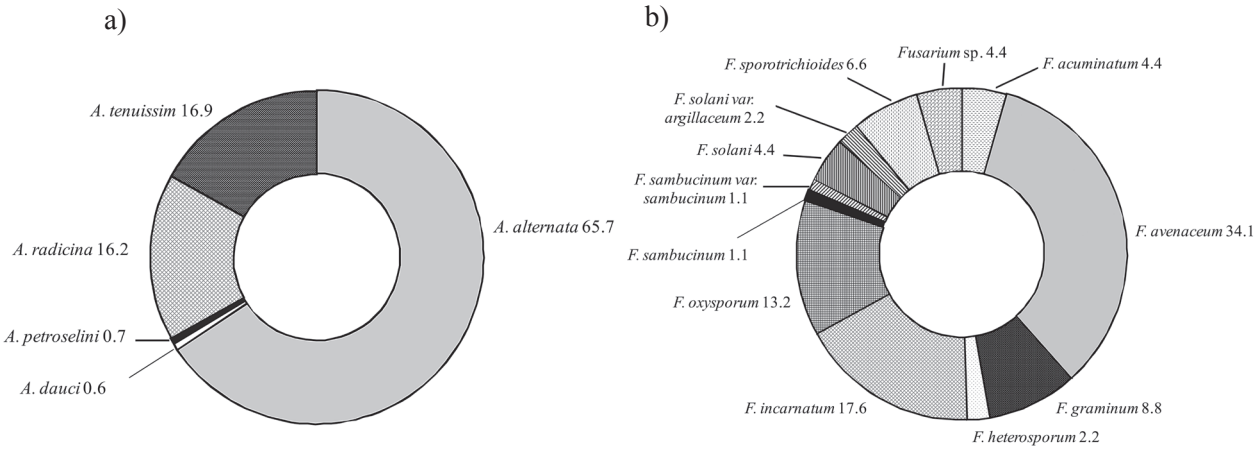

Figure 2. The occurrence of *Alternaria* (a) and *Fusarium* (b) species inside the common caraway seeds (relative density, % among *Alternaria* and *Fusarium* isolates)

These three *Alternaria* species were detected in all years of investigation. Their FO ranged from 0.5% to 84.0% in the seed samples from different growing localities (Tables 2–4). *A. dauci* and *A. petroselini* (FO 0.3%) were recorded, accordingly, in the seeds of 2004 and 2003 harvest years only (Table 1).

The fungi of *Aspergillus* and *Penicillium* genera were of frequent occurrence as well. *Aspergillus* spp. were observed in 38.1% and *Penicillium* spp. in 71.4% of the total common caraway seed samples investigated. Their FO amounted to up to 14.1% and 5.5% in average, whereas in most contaminated seed samples up to 69.5% and 39.0%, respectively (Tables 1–4). *Aspergillus* spp. (FO 22.9%) were most frequent in seed samples of 2003 harvest year, and *Penicillium* spp. (FO 18.0%) in seeds of 2001 harvest year (Fig. 3). The seeds of wild common caraway were most heavily infected by these fungi.

The FO of fungi of genus *Ulocladium* amounted to up to 3.6% in average. They were found in more than one-third of tested common caraway seed samples of 2003 and 2004 harvest years, only. More frequent they were in 2004 (FO 10.9%). Three species of genus *Ulocladium* (*U. atrum*, *U. consortiale* and *U. oudemansii*) were identified inside the common caraway seeds. Last-mentioned prevailed in the seeds of 2004 harvest year. Its FO made up 10.7% in average, however in the most severely infected seeds it amounted to up to 22.5% (Tables 1–4, Fig. 3).

The fungi of *Fusarium* genus were observed in all years of investigation and were detected in almost half of the seed samples tested. Their FO made up 2.8% in average. The most frequent (FO 4.0%) they were inside the seeds of 2002 harvest year although their FO in the seeds from different localities differed significantly (Tables 2–4, Fig. 3). Nine *Fusarium* species (*F. acuminatum*, *F. avenaceum*, *F. graminum*, *F. heterosporum*, *F. incarnatum*, *F. oxysporum*, *F. sambucinum*, *F. solani*, *F. sporotrichioides*) and two varieties (*F. sambucinum* var. *sambucinum*, *F. solani* var. *argillaceum*) were identified inside the common caraway seeds. The most frequent among them were *F. avenaceum* (RD 34.1%), *F. incarnatum* (RD 17.6%), *F. oxysporum* (RD 13.2%), *F. graminum* (RD 8.8%) and *F. porotrichioides* (RD 6.6%) (Fig. 2 b). *F. incarnatum* (FO 1.1%) and *F. sporotrichioides* (FO 1.8%) were most common in seed samples of 2002 harvest year, *F. avenaceum* (FO 1.3%), *F. graminum* (FO 0.4%) and *F. oxysporum* (FO 0.7%) in seed samples of 2003 harvest year. The latter *Fusarium* species was found in this one year only, as well as *F. acuminatum* (FO 0.2%) and *F. sambucinum* (FO 0.2%). The highest species diversity was ascertained in seeds of 2003 and 2004 harvest years: eight and six species, accordingly (Table 1). Only one species – *F. sambucinum* var. *sambucinum* (FO 0.7%) – was found in the internal tissues of seeds of 2001 harvest year. The seeds of cultural caraway were more heavily infected by *Fusarium* spp.

Cladosporium spp. were the most frequent in common caraway seeds of 2004 (FO 4.1%) and 2001 (FO 3.0%) harvest years (Fig. 3). They were found in two-thirds of seed samples tested. Four species of genus *Cladosporium* were identified. *C. herbarum* and *C. cladosporioides* were most common among them. Their FO made up 1.4% and 1.1% in average, respectively, when in the most strongly infected seeds it amounted to up to 7.5% (Tables 1–4).

Table 2. Fungi inside the common caraway seeds of 2001–2002 harvest in various localities

Fungi	Frequency of occurrence %					
	2001			2002		
	I	II	III	IV	V	VI
Acremoniella atra	0	0	0	0	0	3,0
Acremonium strictum	2.0	0	0	0	0	0
Alternaria alternata	0	27.0	11.0	48.0	70.7	51.0
A. radicina	0	33.0	1.0	28.0	21.3	44.0
A. tenuissima	0	11.0	3.0	20.0	46.7	43.0
Arthrinium phaeospermum	0	0	3.0	0	2.7	0
Ascochyta biforae	0	0	0	0	0	2.0
Aspergillus spp.	0	0	18.0	0	0	0
Chaetomium bostrychodes	15.0	0	0	0	0	0
Cladosporium gossypiicola	0	3.0	1.0	0	1.3	0
C. herbarum	5.0	0	0	0	1.3	0
Fusarium avenaceum	0	0	0	2.0	0	0
F. incarnatum	0	0	0	3.0	0	0
F. sambucinum var. *sambucinum*	0	0	1.0	0	0	0
F solani	0	0	0	1.0	0	0
F. sporotrichioides	0	0	0	5.0	0	0
Mycelia sterilia	3.0	4.0	5.0	11.0	2.7	0
Monilia sp.	0	0	1.0	0	0	0
Mortierella sp.	2.0	0	0	0	0	0
Mucor sp.	0	2.0	0	0	0	7.0
Penicillium spp.	9.0	6.0	39.0	0	0	0
Phoma spp.	1.0	5.0	3.0	0	8.0	6.0
Rhizoctonia sp.	1.0	0	1.0	0	0	0
Scytalidium sp.	0	0	0	8.0	0	0
Sclerotinia sclerotiorum	0	0	1.0	0	0	0
Scopulariopsis brevicaulis	0	0	3.0	0	0	0
Scopulariopsis fusca	0	0	1.0	0	0	0
Sordaria fimicola	0	0	0	6.0	2.7	0
Sporotrichum aurantiacum	0	0	0	3.0	6.7	0
Stemphyllium botryosum	0	1.0	1.0	13.0	13.3	8.0
S. sarciniforme	0	0	0	0	1.3	0
Trichoderma viride	0	0	0	0	0	1.0
Trichothecium roseum	0	0	0	0	0	3.0
Volucrispora graminea	0	0	0	0	2.7	0

Note. In 2–6 tables I–XXI – the species, cultivars of common caraway and the localities of seed sampling: I – *Carum carvi,* Vilnius, Pagubė; II – *C. carvi* 'Rekord', trade company "Sėklos"; III – *C. carvi* 'Rekord', trade company "Agrolitpa"; IV – *C. carvi*, Utena distr., Leliūnai; V – *C. carvi*, Vilnius, Naujininkai; VI – *C. carvi* 'Gintaras', Kaunas distr., Noreikiškės; VII – *C. carvi*, Vilnius, Salininkai; VIII – *C. carvi*, Vilnius distr. Rudamina; IX – *C. carvi*, Varėna distr., Marcinkonys; X – *C. carvi*, Biržai distr., Stačkūnai; XI – *C. carvi*, Vilnius distr., Beržiškės; XII – *C. carvi*, Varėna distr.; XIII – *C. carvi* 'Rekord'; Vilnius, Kairėnai; XIV – *C. carvi* 'Rekord', Vilnius, Jeruzalė; XV – *C. carvi* 'Kančevitskij', Ukmergė distr., Juodausiai; XVI – *C. carvi* 'Rekord', Vilnius, Kairėnai; XVII – *C .carvi*, Kaunas distr., Čekiškė; XVIII – *C. carvi*, Šilutė distr.; XIX – *C. carvi*, Vilnius distr., Rykantai; XX – *C. carvi*, Vilnius, Žirmūnai; XXI – *C. carvi*, Raseiniai distr.

Ochrocladosporium elatum (syn. *Cladosporium elatum* (Harz) Nannf.) was recorded in seeds of 2004 harvest year only, however in five seed samples out of the six samples tested. Its FO this year made up 8.1% in average when in most severely infected seeds it amounted to up to 17.5% (Table 4, Fig. 3).

The FO of *Mucor* spp. made up 1.7% in average (Table 1). These fungi infected almost half of the seed samples tested and were observed in all experimental years. However, they were most common inside the cultural caraway seeds of 2002 and 2003 harvest years, when their FO in some localities amounted to up to 7.0% and 11.5%, accordingly (Tables 2–4, Fig. 3).

Sordaria fimicola was found in ten seed samples tested. Its FO amounted to up to 1.7% in average and even up to 10.0% in most strongly infected seeds. This fungus was detected inside the seeds of all harvest years, except 2001 (Tables 1–4, Fig. 3).

Table 3. Fungi inside the common caraway seeds of the 2003 harvest in various localities

Fungi	Frequency of occurrence %								
	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Acremoniella atra	0	0	0	1.5	0	0	0	5.5	0
Acremonium implicatum	0	0	0.5	0	0	0	0	0	0
A. strictum	0	0	0	0	0	0	0	1.5	0
Alternaria alternata	0	0	0	10.0	46.5	21.5	43.5	84.0	3.0
A. petroselini	0	0	0	0	1.5	0	0	4.0	0
A. radicina	0	0.5	0	1.0	12.5	6.5	12.0	6.5	0.5
A. tenuissima	0	0	0	3.0	12.0	5.0	9.0	17.0	0.5
Arthrinium phaeospermum	0	0	0	7.0	3.5	3.0	0.5	2.0	0
Aspergillus spp.	63.0	61.0	69.5	8.5	0	0	0	0	4.0
Ascochyta biforae	0	0	0	0	0	0.5	12.0	0	0
Ascochyta sp.	0	0	0	0	2.5	0	0	0	0
Chaetomium globosum	0	0	0	0	0	4.5	4.0	0.5	0
Cladosporium cladosporioides	0	0	0	0	0	0	0	4.0	0
C. epiphyllum	0	0	0	0.5	0	0	0	0	0
C. herbarum	0	0	0	0	5.0	3.5	7.5	0	0
Cylindrocarpon didymum	0	0	0	0	0	0	0	0.5	0
Geotrichum candidum	0	0	0	2.0	0	0	0	0	0
Fusarium acuminatum	0	0	0	0	0	0	0	2.0	0
F. avenaceum	0	0	0	0	0	0	0	11.5	0
F. graminum	0	0	0	0	0	0	0	3.5	0
F. heterosporum	0	0	0	0	0	0	0	0.5	0
F. incarnatum	0	0	0	0	0	0	0	4.5	0
F. oxysporum	0	0	0	0	0	3.5	0	2.0	0.5
F. sambucinum	0	0	0	0	0	0	0	0.5	0
F. solani	0	0	0	0	0	0	0	1.5	0
Mycelia sterilia	3.0	9.0	1.5	8.5	1.0	5.5	9.0	2.0	5.0
Mucor spp.	0	1.0	0.5	0	0	0.5	0	5.0	11.5
Neofusicoccum mangiferae	0	0	0	1.5	0	0	0	0	0
Penicillium spp.	19.5	8.5	15.0	0.5	2.5	7.0	0.5	0.5	1.5
Phoma sp.	0	0	0	0	0.5	0	0	0	0
Phomopsis diachenii	0	0	0	0	0	0	0	10.5	0.5
Phomopsis sp.	0	0	0	0	0	0	1.5	0	0
Rhizoctonia sp.	0	0	0	0	0	0.5	0	0	0
Sordaria fimicola	0	0	0	10.0	3.0	0.5	6.5	0	0
Sporormiella sp.	0	0	0	0	0	0.5	0	0	0
Sporotrichum aurantiacum	0	0	0	0	3.5	2.5	0	1.0	0
Stemphylium botryosum	0	0	0	0	0.5	0	0	0	0
Trichoderma viride	0	0	0	0	0	4.5	2.0	0	0
Trichothecium roseum	0	0	0	0	0	0	1.5	0	0
Truncatella truncata	0	0	0	0	0.5	0	0	0	0
Ulocladium atrum	0	0	0	0	0	0	0	0.5	0
U. consortiale	0	0	0	0	0.5	4.0	0	4.0	0
Verticillium albo-atrum	0	0	0	3.5	0	0	0	0	0

The fungi of genus *Ascochyta* were isolated from common caraway seeds of all harvest years, except 2001, as well. Their FO made up 1.3% in average. These fungi were most common inside seeds sampled in 2003 (FO 1.7%). One species (*A. biforae*) of this genus was identified. The common caraway 'Rekord' seeds grown in 2003 in Kairėnai were most severely infected by *A. biforae* (FO 12.0%) (Tables 1–4, Fig. 3).

The FO of *Arthrinium phaeospermum* made up 1.4% in average, although in most heavily infected seeds it amounted to up to 7.0%. This fungus was detected in all experimental years in almost half of the common caraway seed samples tested. It was most frequent inside seeds of 2003 harvest year (Tables 1–4, Fig. 3).

Table 4. Fungi inside the common caraway seeds of the 2004 harvest in various localities

Fungi	Frequency of occurrence %					
	XVI	XVII	XVIII	XIX	XX	XXI
Alternaria alternata	84.0	0.5	47.0	28.0	56.7	22.0
A. dauci	1.5	0	3.0	0	0	0
A. radicina	2.0	0.5	3.5	0	11.3	11.0
A. tenuissima	13.0	0.5	6.0	0	8.0	2.0
Arthrinium phaeospermum	0	0	1.0	0	1.3	2.0
Aspergillus spp.	0	1.0	0	0	0	14.0
Ascochyta biforae	3.0	0	0.5	2.0	0	0
Ascochyta sp.	0	0	0	0	0.7	0
Aureobasidium pullulans	0	0	0	14.0	7.3	0
Botrytis cinerea	0	0	0	0	0	3.0
Chaetomium cochliodes	0	0	0	4.0	0	0
Chrysonilia sitophyla	0	0	0	0	0	1.0
Cladosporium cladosporioides	7.5	0	5.5	0	1.3	0
C. herbarum	1.0	0.5	2.0	2.0	0.7	0
Fusarium avenaceum	3.0	0	0	0	0	0
F. graminum	0	0	0	0	0	1.0
F. heterosporum	0	0	0	2.0	0	0
F. incarnatum	2.0	0	0	0	0	0
F. solani var. *argillaceum*	0	0	0	2.0	0.7	0
F. sporotrichioides	0.5	0	0	0	0	0
Fusarium sp.	0.5	0	0	0	2.0	0
Leptosphaeria libanotis	0	0	0	0	0	2.0
Mycelia sterilia	2.5	2.0	3.5	6.0	2.0	22.0
Mucor spp.	0.5	4.5	0	0	0.7	0
Neofusicoccum mangiferae	0	0	0	2.0	0	0
Ochrocladosporium elatum	17.5	0	8.5	2.0	12.0	1.0
Penicillium spp.	0	2.0	0.5	2.0	0	4.0
Phoma anethi	0	0	0	0	0.7	0
Phoma sp.	0	0.5	0	0	0.7	1.0
Phomopsis sp.	1.0	0	0.5	2.0	0	0
Rhizoctonia sp.	0	0	0	0	0	1.0
Scopulariopsis fusca	0	0	0	0	0	2.0
Septoria umbelliferarum	0	0	0.5	0	0	0
Septoria sp.	0.5	0	0	0	0	0
Sordaria fimicola	1.5	0	1.0	2.0	0.7	0
Spilodochium vernoniae	0	0	0	0	0	1.0
Sporotrichum aurantiacum	1.0	0	0	0	0	0
Torula herbarum	1.0	0	0.5	0	0	0
T. ellisii	0	0	2.0	0	0	0
Ulocladium atrum	0.5	0	0	0	0	0
U. consortiale	0.5	0	0	0	0	0
U. oudemansii	22.5	0	18.0	0	10.0	0
Volucrispora graminea	3.5	0	0	0	0	0

Three fungi species of genus *Chaetomium* were recorded inside the common caraway seeds. Their FO made up 1.1% in average. *C. bostrychodes* (FO 15.0%) and *C.cochliodes* (FO 4.0%) were identified in seeds sampled only in one locality in 2001 and 2004, accordingly. *C. globosum* (FO 0.5–4.5%) was found only in seeds of 2003 harvest year sampled in three localities (Tables 1–4, Fig. 3).

The FO of other fungi identified inside the common caraway seeds was below 1.0% (Table 1).

The obtained results showed that the common caraway seeds are heavily infected by fungi. Other researchers also points out a strong fungal contamination of caraway seeds. The study of mycopopulation of caraway seeds in Serbia ascertained fungi belonging to 11 genera and 23 species. *Aspergillus* spp. and *Penicillium* spp. were the dominant contaminants with frequency in total mycopopulation ranging from 57.1% to 35.8%, respectively (Dimić et al., 2008). Machowicz-Stefaniak and Za-

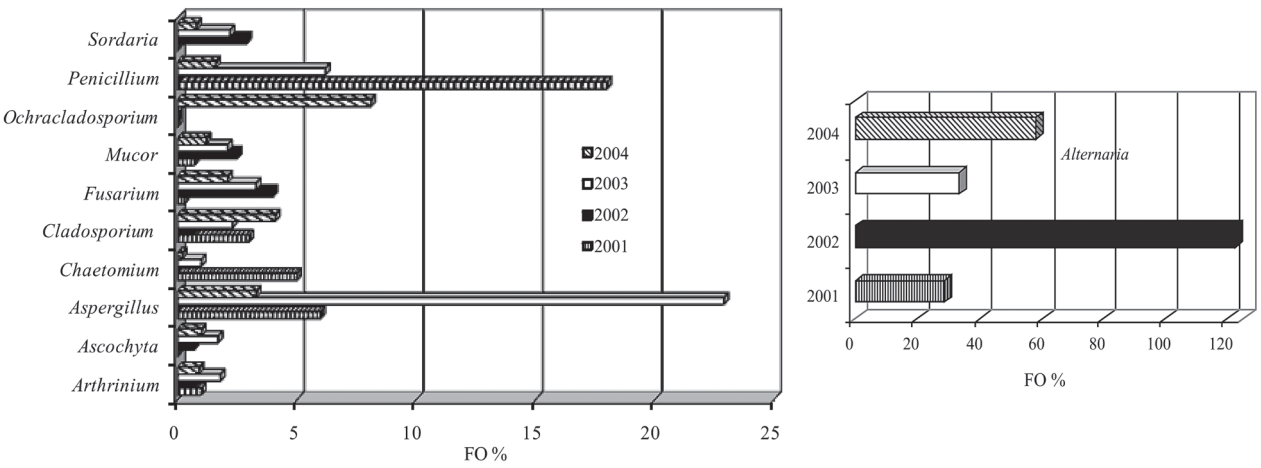

Figure 3. The occurrence of some fungi inside the common caraway seeds in different years (FO exceed 100% as several *Alternaria* species were isolated form the same seed)

lewska (2008) investigating biodiversity of fungi, colonizing different parts of caraway, found a complex of fungi, including *Alternaria* spp., *Fusarium* spp., *Septoria carvi*, *Botrytis cinerea*, *Phoma exigua* var. *exigua*, *Sclerotinia sclerotiorum*, *Cladosporium cladosporioides*, *Epicoccum purpu-rascens* on its schizocarps. Odstričilová (2007) notes, that *Alternaria alternata* and *Cladosporium cladosporioides* are most common fungi on caraway seeds in Czech Republic, although *Cladosporium herbarum*, *Fusarium avenaceum*, *F. culmorum*, *F. equiseti*, *Epicoccum nigrum*, *Trichothecium roseum* and *Aureobasidium* sp. were detected as well. Ostry et al. (2002) observed that caraway seeds are strongly contaminated by toxigenic strains of *Aspergillus* spp. The study of caraway seeds mycoflora in India, demonstrates that *Aspergilus* spp., *Alternaria alternata*, *Fusarium moniliforme*, *F. oxysporum*, *Helminthosporium tetramera*, *Rhizoctonia bataticola* and *Tubercularia vulgaris* prevailed in it (Sumanth et al., 2010). Most of the mentioned fungi were identified inside common caraway seeds during our investigation as well.

The study evidenced that fungal complexes identified inside the cultural and wild caraway seeds have very high qualitative (Sorenson's index (SI) 66.1%) and high quantitative (SI 52.0%) similarity, however, they significantly differ between years and localities. The comparison of fungal complexes inside the common caraway seeds sampled in various localities shows, that most of them have low qualitative and quantitative similarity (SI ranged between 0–38.5%). The higher amount (71.4–89.0%) of fungal complexes detected inside the wild caraway seeds have low similarity, compared with cultural caraway. Very high qualitative (SI 60.0–88.9%) and quantitative (SI 71.4–92.2%) similarity have only 3.3% and 4.4% of fungal complexes inside the wild caraway seeds, accordingly. The 9.5% of fungal complexes inside the cultural caraway seeds shows very high qualitative and quantitative similarity (SI ranged between 60.0–66.7%) (Tables 5–6). The reason of greater similarity of fungal complexes inside the cultural caraway seeds, probably, could be the similarity of conditions under which caraway is cultivated. The conditions in which wild caraway grows are more diverse.

Table 5. The qualitative (a) and quantitative (b) similarity of fungal complexes inside the cultural caraway seeds grown in different localities

a)

Locality	Sorenson's index %					
	II	III	VI	XIII	XIV	XV
III	**66.7**					
VI	**60.0**	37.1				
XIII	36.4	34.5	54.5			
XIV	24.4	20.8	24.4	27.9		
XV	55.6	40.0	44.4	40.0	35.9	
XVI	25.6	17.4	20.5	34.1	33.3	21.6

b)

Locality	Sorenson's index %					
	II	III	VI	XIII	XIV	XV
III	21.6					
VI	**60.8**	14.6				
XIII	43.0	11.6	53.7			
XIV	25.2	8.9	42.9	45.1		
XV	19.1	27.7	14.2	7.3	11.1	
XVI	21.3	8.3	33.1	45.2	**65.5**	4.7

Fungal complexes inside the common caraway seeds of 2001 and 2003 as well as 2001 and 2004 harvest year had the least similarity both qualitative (SI 36.9% and 36.4%) and quantitative (SI 16.1% and 24.1%, accordingly). The highest qualitative (SI 52.9%) and quantitative (SI 49.4%)

similarity of fungal complexes was established in common caraway seeds of 2003 and 2004 harvest years. The fungal complexes in seeds of 2002 and 2003 harvest years had very high qualitative similarity (SI 56.7%); however low quantitative similarity (SI 37.4%) (Table 7). According to the data of Lithuanian Hydrometeorology Station the second half of June in 2003 and 2004, when cool and rainy weather prevailed, were quite comparable and this could determine the high similarity among fungal complexes of these years. The June in 2001 when warm and dry periods alternated with cold and wet ones significantly varied from June in 2003 and 2004. This could lead to the diversity of fungal complexes inside the common caraway seeds. The climatic conditions of the year during seed ripening as well as microclimate of the locality where caraway was grown are influential factors in the formation of fungal complexes in common caraway seeds.

Table 6. The qualitative (a) and quantitative (b) similarity of fungal complexes inside the wild caraway seeds grown in different localities

a)

Locality	Sorenson's index %												
	I	IV	V	VII	VIII	IX	XX	XI	XII	XVII	XVIII	XIX	XX
IV	8.7												
V	28.6	53.8											
VII	16.7	11.8	0										
VIII	15.4	11.1	0	57.1									
IX	14.3	10.5	0	50.0	**88.9**								
XX	10.0	40.0	34.8	28.6	40.0	37.5							
XI	26.1	42.9	**61.5**	11.8	22.2	21.1	48.0						
XII	24.0	33.3	42.9	10.5	30.0	28.6	44.4	**60.0**					
XVII	47.1	27.3	50.0	36.4	50.0	46.2	31.6	36.4	33.3				
XVIII	24.0	26.7	42.9	10.5	10.0	9.5	29.6	33.3	37.5	41.7			
XIX	28.6	23.1	25.0	13.3	12.5	11.8	26.1	23.1	28.6	30.0	50.0		
XX	24.0	26.7	50.0	0	10.0	9.5	22.2	40.0	43.8	50.0	56.3	42.9	
XXI	34.8	21.4	38.5	28.6	22.2	21.1	32.0	21.4	26.7	54.5	33.3	15.4	33.3

b)

Locality	Sorenson's index %												
	I	IV	V	VII	VIII	IX	XX	XI	XII	XVII	XVIII	XIX	XX
IV	3.2												
V	4.6	**71.4**											
VII	7.7	3.8	0										
VIII	8.5	3.9	0	**87.9**									
IX	7.5	1.9	0	**92.2**	**85.9**								
XX	1.4	31.8	24.5	17,1	26.0	14.8							
XI	10.8	51.0	59.1	4.2	4.3	4.1	30.4						
XII	16.3	44.7	46.1	13.1	17.7	11.0	53.3	53.3					
XVII	16.4	7.0	7.6	6.2	8.8	7.1	6.0	7.4	8.9				
XVIII	10.6	19.9	21.0	0.7	0.7	0.7	9.9	15.1	15.4	11.8			
XIX	16.9	5.9	5.1	1.0	1.1	1.0	4.6	4.3	5.2	22.7	13.4		
XX	7.8	27.7	31.1	0	0.8	0.8	9.0	14.1	14.3	14.2	59.1	25.2	
XXI	12.3	19.3	16.8	20.9	16.8	20.7	18.3	1.0	11.0	13.2	14.6	4.1	20.5

Table 7. The qualitative and quantitative similarity of fungal complexes inside the common caraway seeds of different harvest years

Year of harvest	Qualitative similarity			Quantitative similarity		
	Sorenson's index %					
	2001	2002	2003	2001	2002	2003
2002	43.5			32.6		
2003	36.9	**56.7**		16.1	37.4	
2004	36.4	47.1	**52.9**	24.1	41.1	**49.4**

Our research has shown that saprotrophes dominated in the internal mycobiota of common caraway seeds. *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Fusarium* spp., *Cladosporium* spp., *Ulocladium* spp. and others are active producers of secondary metabolites. They are responsible for mycotoxin accumulation in seeds and quality of seeds used as raw material in pharmaceutical and food industry. The fungal invasion increases toxin production in seeds and seed toxicity. The number of potential pathogens was observed inside the

common caraway seeds as well. *Alternaria alternata*, *Ascochyta biforae*, *Botrytis cinerea*, *Fusarium* spp., *Phoma* spp., *Phomopsis* spp., *Rhizoctonia* sp., *Verticillium albo-atrum* were of the most frequent occurrence among them. They can injure the common caraway umbels, leaves, stems, roots and are named as caraway disease agents in many references (Gabler, 2001; Pearse, 2002; Mazur, Nawrocki, 2004; Machowicz-Stefaniak, 2009). The potential pathogens inside common caraway seeds, being the reason of diseases transmission in crops, can cause significant damage to common caraway seed yield and quality in spite of not very frequent occurrence. Favourable climatic conditions may increase their development and the harm they cause. In order to minimize the damage of fungi and mycotoxins, which they produce, for human health the efforts to grow and preserve healthy yield of caraway seeds must be undertaken. Improving the conditions of caraway seed growing, storage, transport and continuous mycological and toxicological control prior to food processing is necessary. Seed health testing may detect the presence of disease transmitting and toxin producing fungi in seeds, which would aid in reducing the risk of using infected seed lots as sowing material or food and medicine.

Conclusions

1. The fungi of 55 species, two varieties and 39 genera, belonging to *Ascomycota*, *Basidiomycota* and *Zygomycota* phyla, *Sordariomycetes*, *Dothideomycetes*, *Leotiomycetes*, *Eurotiomycetes*, *Sacharomycetes*, *Agaricomycetes* and *Incertae sedis* classes were identified inside the common caraway seeds. The micromycetes of genus *Alternaria* dominated in the internal mycobiota of investigated seeds. They made up 48.1% of the total isolate amount and the frequency of their occurrence amounted to 47.1%.

2. *Alternaria alternata*, *Ascochyta biforae*, *Botrytis cinerea*, *Fusarium* spp., *Phoma* spp., *Phomopsis* spp., *Rhizoctonia* sp., *Verticillium alboatrum* – the agents of plant spots, wilts and rots were observed inside the common caraway seeds, although the saprotrophes, potential producers of mycotoxins, prevailed.

3. The fungal complexes identified inside the cultural and wild caraway seeds have very high qualitative and high quantitative similarity; however, they significantly vary between years and localities. The greater similarity was ascertained among fungal complexes, identified inside the seeds of cultural caraway, compared with fungal complexes in wild caraway seeds.

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Laukinio ir kultūrinio paprastojo kmyno (*Carum carvi* L.) sėklų vidinė mikobiota

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Santrauka

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

Paprastojo kmyno sėklų viduje buvo aptikti ir identifikuoti 55 rūšių, 2 varietetų ir 39 genčių grybai, priklausantys *Ascomycota*, *Basidiomycota ir Zygomycota* skyriams, *Sordariomycetes*, *Dothideomycetes*, *Leotiomycetes*, *Eurotiomycetes*, *Sacharomycetes*, *Agaricomycetes* ir *Incertae sedis* klasėms. Tirtų sėklų vidinėje mikobiotoje vyravo *Alternaria* genties mikromicetai, kurie sudarė 48,1 % visų izoliatų, o jų aptikimo dažnis siekė 47,1 %. Paprastojo kmyno sėklų viduje aptikti *Alternaria alternata*, *Ascochyta biforae*, *Botrytis cinerea*, *Fusarium* spp., *Phoma* spp., *Phomopsis* spp., *Rhizoctonia* sp., *Verticillium alboatrum* augalų dėmėtligių, vytulių, puvinių sukėlėjai, tačiau sėklose vyravo saprotrofai *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Ulocladium* spp. ir kiti potencialūs mikotoksinų producentai. Laukinių ir kultūrinių kmynų sėklų viduje nustatyti grybų kompleksai turi labai didelį kokybinį bei didelį kiekybinį panašumą, tačiau gerokai skiriasi įvairių augaviečių ir skirtingų derliaus metų sėklose. Didesnis panašumas nustatytas tarp grybų kompleksų, identifikuotų kultūrinių kmynų sėklų viduje, palyginti su laukinių kmynų sėklose aptiktais grybų kompleksais.

Reikšminiai žodžiai: *Carum carvi*, grybai, sėklos, aptikimo dažnis, santykinis tankis.