DE GRUYTER OPEN

herba polonica
From Botanical to Medical Research
Vol. 61 No. 4 2015

DOI: 10.1515/hepo-2015-0027

EXPERIMENTAL PAPER

Incubation methods for the detection of fungi associated with caraway (*Carum carvi* L.) seeds

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S u m m a r y

Introduction: Infestation with fungi may significantly affect the quality of seeds. However, there is no standard method for caraway (*Carum carvi* L.) seed health testing. **Objective:** The aim of the present study was to determine the most efficient method of the detection of fungi associatyed with caraway seeds. **Methods:** Seven incubation methods for evaluation of health of these seeds were compared: deep freeze blotter test, blotter test with mannitol, blotter test with polyethylene glycol, agar tests on potato-dextrose-agar (PDA) and on reduced PDA (RPDA) without seed disinfection, and agar tests on PDA and RPDA after seed disinfection. The evaluation was performed after 10 and 14 days of incubation. **Results:** Thirty two fungal genera were associated with the seeds. *Alternaria alternata*, *Cladosporium* spp. and *Rhizopus nigricans* were identified most frequently. Prolongation of incubation time favoured growth of *Fusarium* spp. and *R. nigricans* to the highest extent. **Conclusions:** The greatest seed infestation with fungi, especially *Alternaria* spp., was observed in the deep freeze blotter test followed by the blotter test with mannitol. Both of them could be recommended for further study on caraway seed health testing.

Key words: *caraway seeds, blotter test, agar test, seed health*

INTRODUCTION

Caraway (*Carum carvi* L.) schizocarps (in practice called as a seed) are used in meat, food and distillery industries due to their intense and pleasant taste

and flavour [1]. Moreover, caraway has a long history as a medicinal plant [2-11]. In ethnopharmacology, caraway is used for digestive problems, such as heartburn, bloating, gas, appetite loss, mild spasms of stomach and intestines. Caraway oil helps cough up phlegm, improves control of urination and relieve constipation, start menstruation and relieve menstrual cramps. The nursing mothers use it to increase the flow of breast milk. The essential oil of *C. carvi* possess strong antifungal and antibacterial potential [2-5]. The main components of caraway essential oil, i.e. carvone, limonene, germacrene D, and *trans*-dihydrocarvone showed activity against wide range of bacteria and fungi [4, 5]. Moreover, carvone and limonene have been investigated as a potential cancer chemoprotective agents [6]. It was found that caraway might inhibit tumorigenesis of colon cancer in rats – caraway dietary supplementation significantly reduced aberrant crypt foci development and decreased the levels of fecal bile acids, neutral sterols, and tissue alkaline phosphatase activities [7, 8]. The aqueous extracts of *C. carvi* exhibited a potent anti-hyperglycaemic action and strong diuretic activity [9, 10]. Moreover, caraway essential oil strongly inhibited lipid peroxidation, showing antioxidative and hematoprotective potential [11]. Despite medical activity, carvone is also used as fragrance and flavour, potato sprouting inhibitor and biochemical environmental indicator [4, 12].

For use in pharmacy and food industry, a high quality of herbal material is required. However, many pathogenic, potentially pathogenic and saprotrophic fungi have been identified on caraway seeds. *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea* Pers. ex Pers., *Cladosporium* spp., *Colletotrichum* spp., *Epicoccum nigrum* Link, *Fusarium* spp., *Penicillium* spp., *Phoma* spp. and *Septoria* spp. are the most common ones [13-23]. Pathogenicity against caraway has been proved for: *Alternaria alternata* (Fr.) Keissl. [14, 18], *A. tenuissima* (Kunze) Wiltshire [18], *B. cinerea* [14], *Cladosporium cladosporioides* (Fresen.) G.A. de Vries [13], *Colletotrichum dematium* (Fr.) [20, 22], *C. gleosporioides* (Penz.) Sacc. [21], *Fusarium equiseti* (Corda) Sacc. [23], *Sclerotinia sclerotiorum* Lib. de Bary [13] and *Septoria carvi* Syd. [13]. Many of these fungi, especially from *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* genera, are potential mycotoxin producers [24]. In addition, infestation of seeds with *Aspergillus flavus* Link., *A. niger* Tiegh. and *Fusarium verticillioides* (Sacc.) Nirenberg may result in a reduction of protein, carbohydrates and total oils and in an increase of fatty acids content in caraway seeds during storage [25]. Furthermore, fungi occurring on seeds may significantly affect their germination, vigour and plants emergence, and can be a primary source of inoculum causing diseases in the future plants [26]. However, there is no standard method for caraway seed health testing. The incubation methods, i.e. agar and blotter tests, simple and relatively cheap, are still frequently used for detection and identification of many seed-associated fungi [26, 27]. Chemicals secreted during seed germination may decrease the growth of fungi in blotter tests and make their detection and identification

difficult. Therefore, different modifications of these tests, such as deep freezing or water restriction technique have been used to overcome this difficulty [26, 28-30]. The other problem in incubation tests may be a lack of sporulation of fungi, which is essential for their identification, if based on morphology. For this reason, the media with low content of carbohydrates, such as reduced potato dextrose agar, have been proposed to stimulate production of spores [31]. Significant problems in agar tests are also fast growing seed contaminants, such as *Rhizopus nigricans* Ehrenb. which inhibits the growth of pathogens. The solution of this problem may be seed disinfection, which removes those microorganisms from seed surface [32]

The aim of the experiment was to determine the most efficient method of the detection of fungi associated with caraway seeds.

MATERIAL AND METHODS

Six lots of caraway seeds produced in Poland in the years 2009-2010 were used in the experiment. The lots were obtained from: Astex company (Masovia) – lot I, W. Legutko Seed Company (Greater Poland) – lots II and III, Vilmorin Company (Greater Poland) – lot IV, Polan Kraków Company (Lesser Poland) – lot V and Herbanordpol Gdańsk Company (Pomerania) – lot VI.

For each lot, 200 seeds (five replicates of 40 seeds) were tested with each of seven methods.

Un-disinfected seeds were placed on six layers of blotter:

I. soaked with distilled water in case of deep freeze blotter test (DFBT),
II. soaked with 5 ml of mannitol (Sigma, osmotic potential –1.5 MPa, 112.2 g mannitol l^{-1}, sterile distilled water) in case of blotter test with mannitol (BT+Mn) [33],
III. soaked with 5 ml of polyethylene glycol (PEG 8000, osmotic potential -1.0 MPa, 284 g PEG l^{-1}, sterile distilled water) in case of blotter test with polyethylene glycol (BT+PEG) [34],

or on the surface of:

IV. potato-dextrose-agar (PDA; Merck, 39 PDA g l^{-1} sterile distilled water) supplemented with 100 ppm streptomycin sulfate (Sigma) in case of PDA test,
V. reduced potato-dextrose-agar (RPDA; Merck, 10 g agar extra pure l^{-1}, PDA 12 g l^{-1} sterile distilled water) supplemented with 100 ppm streptomycin sulfate in case of RPDA test.

Disinfected seeds were placed on the surface of:

VI. potato-dextrose-agar in case of PDA test with disinfected seeds (PDA+Cl),
VII. reduced potato-dextrose-agar in case of RPDA test with disinfected seeds (RPDA+Cl).

Seeds were disinfected in 1.0% aqueous solution of sodium hypochlorite (NaClO) for 10 min, rinsed three times in sterile distilled water and dried with sterile blotter. In DFBT test seeds were initially incubated at 20°C for 36 h in darkness, frozen at -20°C for 20 h, and subsequently incubated at 20°C under 12 h NUV light/12 h darkness cycle for 6 and 10 days. In methods II-VII seeds were incubated at 20°C under 12 h NUV light/12 h darkness cycle for 10 and 14 days. The osmotic potentials of mannitol and polyethylene glycol were determined in a preliminary experiment.

After incubation, in blotter tests seeds were examined under stereomicroscope (magnification ×50) for fungal growth and sporulation, whereas, in agar tests seeds were primarily examined with bare eye for fungal colonies and next under stereomicroscope for fungal sporulation, if necessary [35-38].

The results obtained, presented as percentage of seeds infested with individual fungi, were compared by means of two-way analysis of variance. Duncan's multiple range test was applied to estimate the differences between the means at a level $\alpha=0.05$.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

The lots differed significantly in terms of the number of fungi detected and percentage of infested seeds. Only: *A. alternata*, *Aureobasidium* sp., *Cladosporium* spp., *E. nigrum*, *Fusarium* spp., *Mucor* sp., *Penicillium* spp., *R. nigricans*, *Ulocladium* spp. and *Verticillium* sp. were found in all tested lots (tab. 1). *Alternaria alternata*, *Cladosporium* spp. and *R. nigricans* were eudominant species with colonization frequency ≥10%, according to Tischler's scale for species dominance [39]. Dominant species with colonization frequency 5–10% were not recorded. Subdominant fungi with colonization frequency of 2–5% were: *Aureobasidium* sp., *Cephalosporium* sp., *Colletotrichum* sp., *Gonatobotrys simplex* Corda, *Fusarium* spp., *Penicillium* spp. and *Trichothecium roseum* Link. *Epicoccum nigrum*, *Phoma* spp., *Sordaria fimicola* Ces. & De Not, *Stemphylium botryosum* Wallr. and *Verticillium* sp. belonged to recedent species with colonization frequency of 1–2%. Other fungi, i.e.: *Alternaria dauci* J.W Groves & Skolko, *A. radicina* Meier, Drechsler & E.D. Eddy, *Acremoniella atra* (Corda) Sacc., *Ascochyta* sp., *Aspergillus* spp., *B. cinerea*, *Bipolaris* spp., *Chaetomium* spp., *Dendryphion comosum* Wallr., *Ulocladium* spp., *Mortierella* sp., *Mucor* spp., *Myrothecium roridum* Tode, *Nigrospora* sp., *Papulaspora* sp., *Sordaria tetraspora* G. Winter, *Sporotrichum* sp., *Stachybotrys atra* Corda, *Thamnidium* sp. and *Torula* sp. were classified as subrecedent species with colonization frequency of 0–1%. They often infested only one lot and were detected with one method (tab. 1). The effects of different methods on the detection of frequently identified fungi as well as potential pathogens and/or mycotoxin producers on caraway seeds are presented in tables 2–5, following the order of their colonization frequency.

Table 1.

Detection of fungi associated with caraway seeds by incubation methods

Fungus	Number of infested lots	Method													
		DFBT 10*	DFBT 14	BT+Mn 10	BT+Mn 14	BT+PEG 10	BT+PEG 14	PDA 10	PDA 14	RPDA 10	RPDA 14	PDA+Cl 10	PDA+Cl 14	RPDA+Cl 10	RPDA+Cl 14
Alternaria alternata	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aureobasidium sp.	6	–	–	–	–	–	–	+	+	+	+	+	+	+	+
Cladosporium spp.	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epicoccum nigrum	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fusarium spp.	6	+	+	+	+	–	–	+	+	+	+	+	+	+	+
Mucor spp.	6	–	–	–	–	–	–	–	–	+	+	–	–	–	–
Penicillium spp.	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhizopus nigricans	6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ulocladium sp.	6	+	+	–	–	–	–	+	+	+	+	–	–	–	–
Verticillium sp.	6	+	+	+	+	+	+	+	+	+	+	–	+	–	–
Alternaria radicina	5	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Aspergillus spp.	5	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Bipolaris spp.	5	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Cephalosporium sp.	5	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Gonatobotrys simplex	5	+	+	+	+	+	+	+	+	+	+	–	–	–	–
Phoma spp.	5	+	+	+	+	+	+	+	+	–	–	+	+	+	+
Sordaria fimicola	5	–	+	–	–	–	–	+	+	+	+	+	+	+	+
Stemphylium botryosum	5	+	+	+	+	–	–	+	+	+	+	+	+	–	–
Trichothecium roseum	5	+	+	+	+	+	+	+	+	+	+	–	–	+	+
Botrytis cinerea	4	+	+	+	+	+	+	+	+	+	+	–	–	+	+
Chaetomium spp.	4	–	+	+	+	–	–	+	+	+	+	+	+	–	+
Papulaspora sp.	4	–	–	–	–	–	–	+	+	+	+	+	+	+	+
Colletotrichum sp.	3	+	+	+	+	+	+	–	–	+	+	–	–	+	+
Dendryphion comosum	3	–	–	–	–	–	–	+	+	–	+	–	–	–	–
Nigrospora sp.	3	–	–	–	–	–	–	–	–	+	+	+	+	–	–
Acremoniella atra	2	–	–	–	–	–	–	–	–	+	+	–	–	–	–
Alternaria dauci	2	+	+	+	+	–	–	–	–	–	+	–	–	–	–
Torula herbarum	2	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Ascochyta sp.	1	–	–	+	+	–	–	–	–	–	–	–	–	–	–
Mortierella sp.	1	–	–	–	–	–	–	–	–	+	+	–	–	–	–
Myrothecium roridum	1	–	–	–	–	–	–	–	+	+	+	–	–	–	–
Sordaria tetraspora	1	–	–	–	–	–	–	–	+	–	–	–	–	–	–

Fungus	Number of infested lots	Method													
		DFBT 10*	DFBT 14	BT+Mn 10	BT+Mn 14	BT+PEG 10	BT+PEG 14	PDA 10	PDA 14	RPDA 10	RPDA 14	PDA+Cl 10	PDA+Cl 14	RPDA+Cl 10	RPDA+Cl 14
Sporotrichum sp.	1	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Stachybotrys atra	1	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Thamnidium sp.	1	–	–	–	–	–	–	–	–	–	–	+	–	–	–

* DFBT 10, DFBT 14 – deep freeze blotter test, incubation for 10 and 14 day, respectively; BT+Mn 10, BT+Mn 14 – blotter test with mannitol, incubation for 10 and 14 day, respectively; BT+PEG 10, BT+PEG 14 – blotter test with polyethylene glycol, incubation for 10 and 14 day, respectively; PDA 10, PDA 14 – agar test on potato-dextrose-agar, incubation for 10 and 14 day, respectively; RPDA 10, RPDA 14 – agar test on reduced potato-dextrose-agar, incubation for 10 and 14 day, respectively; PDA+Cl 10, PDA+Cl 14 – agar test on potato-dextrose-agar after seed disinfection, incubation for 10 and 14 day, respectively; RPDA+Cl 10, RPDA+Cl 14 – agar test on reduced potato-dextrose-agar after seed disinfection, incubation for 10 and 14 day, respectively.

Table 2.

Effects of different methods on the detection of eudominant and subdominant fungi on caraway seeds – means for methods

Method	Percentage of infested seeds				
	Eudominants			Subdominants	
	Alternaria alternata	*Rhizopus nigricans*	*Cladosporium* spp.	*Trichothecium roseum*	*Colletotrichum* spp.
DFBT 10	60.0 ef*	1.4 a	15.3 e	11.9 e	12.8 c
DFBT 14	63.3 f	1.4 a	17.7 e	14.4 e	13.7 c
BT+Mn 10	57.5 e	7.1 b	27.3 fg	3.1 c	13.3 c
BT+Mn 14	59.4 ef	9.3 b	30.4 fg	3.3 c	13.3 c
BT+PEG 10	39.3 c	9.2 b	27.1 f	0.8 b	3.0 b
BT+PEG 14	41.4 c	10.3 b	32.1 g	1.0 b	4.3 b
PDA 10	41.5 c	33.7 d	5.4 c	5.9 c	0 a
PDA 14	44.4 c	53.3 f	7.1 c	6.1 c	0 a
RPDA 10	52.2 d	36.0 de	9.1 d	9.1 d	0.8 a
RPDA 14	54.7 d	45.0 ef	11.7 d	9.8 d	0.8 a
PDA+Cl 10	19.6 a	23.5 c	0.1 a	0 a	0 a
PDA+Cl 14	21.9 ab	69.1 g	0.2 a	0 a	0 a
RPDA+Cl 10	21.8 ab	53.0 f	1.2 ab	0.1 ab	0.2 a
RPDA+Cl 14	23.4 b	67.3 g	1.5 b	0.1 ab	0.2 a
Maximum infestation in the lots (lot number)	4.0(I)-100.0(IV)	36.5(IV)-95.0(I)	4.0(V)-61.5(I)	0.5(V,VI)-59.0(IV)	1.0(I)-39.0(VI)
Frequency of seed colonisation (%)	42.8	29.9	13.21	4.6	4.4

* Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (α=0.05). For further explanation – see table 1.

Table 3.

Effects of different methods on the detection of subdominant fungi on caraway seeds – means for methods

Method	Percentage of infested seeds				
	Penicillium spp.	*Aureobasidium* sp.	*Gonatobotrys simplex*	*Fusarium* spp.	*Cephalosporium* spp.
DFBT 10	1.3 a*	0 a	4.4 c	2.7 b–d	6.0 cd
DFBT 14	1.7 a	0 a	5.1 c	5.4 gh	8.3 de
BT+Mn 10	3.8 b–d	0 a	1.2 b	0.7 ab	0 ab
BT+Mn 14	4.3 b–d	0 a	1.4 b	0.9 a–c	0.5 ab
BT+PEG 10	6.3 de	0 a	0.1 a	0 a	0.1 a
BT+PEG 14	7.8 e	0 a	0.3 a	0 a	1.0 b
PDA 10	4.8 b–d	8.2 d	5.8 cd	3.9 e–g	1.0 ab
PDA 14	5.3 c–e	8.2 d	6.7 d	6.5 hi	5.4 c
RPDA 10	4.5 b	7.3 d	5.4 cd	3.4 e–g	6.4 cd
RPDA 14	4.7 bc	9.5 d	9.9 e	7.8 i	10.0 e
PDA+Cl 10	0.5 a	2.0 b	0 a	1.5 c–e	0.1 ab
PDA+Cl 14	0.8 a	2.3 bc	0 a	3.5 fg	0.1 ab
RPDA+Cl 10	0.8 a	3.6 c	0 a	0.8 a–c	0 a
RPDA+Cl 14	1.2 a	3.8 c	0 a	1.8 d–f	0 a
Maximum infestation in the lots (lot number)	0.5(IV)–17.5(III)	0.5(I)–51.0(II)	0.5(VI)–45.0(IV)	6.0(I,V)–22.5(II)	0.5(V)–26.0(IV)
Frequency of seed colonisation (%)	3.4	3.2	2.9	2.8	2.7

* Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (α=0.05). For further explanation – see table 1.

Table 4.

Effects of different methods on the detection of recedent fungi on caraway seeds – means for methods

Method	Percentage of infested seeds				
	Sordaria fimicola	*Epicoccum nigrum*	*Verticillium* spp.	*Phoma* spp.	*Stemphylium botryosum*
DFBT 10	0 a*	2.8 c	3.9 f	0.5 bc	3.7 c
DFBT 14	0.1 a	4.0 d	5.3 g	0.6 c	4.6 c
BT+Mn 10	0 a	0.7 ab	2.3 ef	6.5 d	1.8 b
BT+Mn 14	0 a	1.1 b	2.3 ef	6.6 d	2.0 b
BT+PEG 10	0 a	0.4 ab	0.3 ab	0.6 bc	0 a
BT+PEG 14	0 a	0.5 ab	0.6 a–c	0.8 c	0 a
PDA 10	3.3 b	2.8 cd	0.6 bc	0.2 ab	0.1 a
PDA 14	4.3 b	3.3 cd	0.6 bc	0.2 ab	0.1 a
RPDA 10	1.5 ab	2.3 c	0.8 cd	0 a	1.2 b

Method	Percentage of infested seeds				
	Sordaria fimicola	*Epicoccum nigrum*	*Verticillium* spp.	*Phoma* spp.	*Stemphylium botryosum*
RPDA 14	1.5 ab	2.4 cd	1.6 de	0 a	1.2 b
PDA+Cl 10	3.0 b	0.4 ab	0 a	0.1 ab	0.1 a
PDA+Cl 14	3.2 b	0.5 ab	0.1 ab	0.1 ab	0.1 a
RPDA+Cl 10	4.4 b	0.3 a	0 a	0.1 ab	0 a
RPDA+Cl 14	4.7 b	0.4 a	0 a	0.1 ab	0 a
Maximum infestation in the lots (lot number)	1.0(II)–14.0(VI)	1.0(I)–10.5(II)	0.5(VI)–26.0(II)	0.5(IV,VI)–33.0(II)	0.5(II)–8.5(IV)
Frequency of seed colonisation (%)	1.8	1.5	1.3	1.2	1.1

* Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (α=0.05). For further explanation – see table 1.

Table 5.

Effects of different methods on the detection of selected subrecedent fungi on caraway seeds – means for methods

Method	Percentage of infested seeds			
	Botrytis cinerea	*Aspergillus* spp.	*Alternaria radicina*	*Alternaria dauci*
DFBT 10	0.3 a–c*	0 a	1.2 b	1.2 b
DFBT 14	0.3 a–c	0.1 ab	1.7 c	1.7 c
BT+Mn 10	1.0 bc	0.3 ab	0.3 a	0.3 a
BT+Mn 14	1.5 c	0.3 ab	0.4 a	0.4 a
BT+PEG 10	0.1 ab	0.2 ab	0.3 a	0.3 a
BT+PEG 14	0.1 ab	0.4 ab	0.3 a	0.3 a
PDA 10	0.8 a–c	0.3 ab	0.2 a	0.2 a
PDA 14	0.9 a–c	0.3 ab	0.2 a	0.2 a
RPDA 10	1.0 a–c	0.3 ab	0.1 a	0.1 a
RPDA 14	1.6 bc	0.5 ab	0.2 a	0.2 a
PDA+Cl 10	0 a	0.6 a–c	0.1 a	0.1 a
PDA+Cl 14	0 a	0.8 bc	0.1 a	0.1 a
RPDA+Cl 10	1.4 a–c	1.8 cd	0 a	0 a
RPDA+Cl 14	1.4 a–c	3.3 d	0 a	0 a
Maximum infestation in the lots (lot number)	0.5(V)–4.0(II)	0.5(I)–13.0(III)	1.0(I)–3.5(V)	0.5(IV)–2.0(III)
Frequency of seed colonisation (%)	0.7	0.6	0.4	0.2

* Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (α=0.05). For further explanation – see table 1.

Alternaria alternata was usually very abundant on tested seeds. In five of six lots the maximal seed infestation ranged from 50.5% to 100%. Only in lot I, the percentage of seed infested with this fungus did not exceed 4%. Other *Alternaria* species, i.e. *A. dauci* and *A. radicina* were detected sporadically and infected low percentage of seeds. The deep-freeze-blotter test favoured growth of all *Alternaria* species and *A. alternata* was also frequently detected in the blotter test with an addition of mannitol. Moreover, the blotter tests favoured growth and identification of *Cladosporium* spp. (BT+Mn, BT+PEG), *Colletotrichum* sp. (DFBT, BT+Mn), *Phoma* spp. (BT+Mn), *S. botryosum* (DFBT), *T. roseum* (DFBT) and *Verticillium* sp. (DFBT, BT+Mn). On the other hand, agar media positively affected the growth of *R. nigricans* (PDA+Cl, RPDA+Cl, PDA, RPDA), *Aspergillus* spp. (RPDA+Cl), *Aureobasidium* sp. (PDA, RPDA), *G. simplex* (PDA, RPDA) and *S. fimicola* (PDA, PDA+Cl, RPDA+Cl). For other fungi, the results were not as unambiguous. *Botrytis cinerea* was identified in comparable percentages on the seeds in the blotter test with an addition of mannitol as well as in the RPDA test without and after seed disinfection. *Epicoccum nigrum* and *Fusarium* spp. were frequently identified in the DFBT test as well as the PDA and RPDA tests. *Penicillium* spp. were detected in comparable percentages on the seeds in the blotter tests with an addition of PEG and mannitol and in the agar test on PDA. In most cases disinfection resulted in a decrease in seed infestation with fungi in agar tests. It was observed for: *A. alternata*, *Cladosporium* spp., *Aureobasidium* sp., *Cephalosporium* sp., *E. nigrum*, *G. simplex*, *Fusarium* spp., *Penicillium* spp., *T. roseum* and *Verticillium* sp. Prolongation of incubation time, depending on the method, favoured growth of only few species, i.e. *Cladosporium* spp. in the blotter test with an addition of PEG, *R. nigricans* in all agar tests, *A. radicina* in the DFBT, *Cephalosporium* sp. in the PDA and RPDA tests, *E. nigrum* in the DFBT, *G. simplex* in the RPDA test, *Fusarium* spp. in most tests, *Penicillium* spp. in the PDA test and *Verticillium* spp. in the DFBT test.

DISCUSSION

The detection of particular fungi associated with caraway seeds depended on the seed lot and method of testing. To prevent seed germination in blotter tests two techniques were used: deep freezing at -20°C and water restriction, through the use of PEG or mannitol as an alternative. Machado et al. [28-30] found that in general, a water potential of -0.6 to -1.0 MPa produced by application of mannitol, sodium chloride or potassium chloride, effectively reduced radicle protrusion and did not affect fungal growth. Moreover, at potentials from -0.3 to -1.0 MPa the growth of some fungi was even stimulated [29]. In the present experiment the inhibition of seed germination was also observed, however, especially when PEG was used, it was associated with a decrease in seed infestation with fungi. The same effect was observed in our previous experiments – an addition of mannitol stimulated growth of *Alternaria* spp., *B. cinerea*, *Cladosporium* spp. and *Epicoccum*

purpurascens Ehranb. (syn. *E. nigrum*) on zinnia seeds, although, the addition of PEG gave opposite results [40].

In the present experiment, *A. alternata*, *Cladosporium* spp. and *Rhizopus* sp. were the most frequently detected on caraway seeds. High infestation of caraway seeds with *A. alternata* was reported by many authors, regardless of the origin of the seeds and harvest year, which suggest a high ability of this saprotrophic fungus to seed infestation [13, 15, 16, 18]. Odstrčilová [19] found that *A. alternata* and *Cladosporium* spp. prevailed on tested caraway seeds, and *Fusarium* spp., *E. nigrum*, *T. roseum*, *Mucor* sp. and *Penicillium* spp. were isolated sporadically. Infestation of caraway seeds with *Alternaria* spp. as the potential producers of mycotoxins, such as alternariol, alternariol monomethyl ether, altenuene, altertoxins I, II and III, and tenuazonic acid, seems to be particularly hazardous [24]. Investigations have shown that many *Alternaria* species as well as their metabolites have been proven to exert cytotoxic, genotoxic and mutagenic effects on animals and humans [41]. *Alternaria* species (including *A. alternata*, *A. dauci*, *A. petroselini* and *A. radicina*) if occur on seeds of several *Apiaceae* species, i.e. carrot, parsley, parsnip and celery, can reduce their germination [42]. Therefore, inhibition of germination of caraway seeds colonized by *Alternaria* spp. can be expected.

Blotter tests, especially the deep freeze blotter test, favoured growth and detection of all *Alternaria* species, i.e. *A. alternata*, *A. dauci* and *A. radicina*, however, last two pathogens infected much smaller number of seeds. Moreover, high percentage of seeds infested with *A. alternata* was detected in the blotter test with an addition of mannitol. The deep freeze blotter test is recommended by the International Seed Testing Association (ISTA) for identification of *A. dauci* and *A. radicina* on seeds of carrot which is the plant closely related to caraway [43, 44]. The high effectiveness of the blotter test with an addition of mannitol in detection of *Alternaria* spp. on zinnia seeds was reported by Szopińska et al. [40].

The fungi from *Colletotrichum* genus were detected only in three of six lots in the present experiment. However, the maximum seed infestation in one of these lots (VI) reached 39.0%. The highest numbers of seeds infested with these fungi were observed if the blotter tests, i.e. the deep-freeze blotter test and the blotter test with an addition of mannitol, were used. Further studies on identification of these fungi on caraway seeds seems to be essential, because *C. dematium* and *C. gloeosporioides* have been reported as potentially pathogenic to this plant [20-22]. Zalewska [22] observed that tested strains of *C. dematium* caused necrosis and dieback of caraway seedlings in laboratory conditions. Moreover, the pathogenic *Colletotrichum* species can cause anthracnose in many plants [20].

The deep freeze blotter test and potato dextrose agar media were the most effective ones in detection of *Fusarium* spp. on non-disinfected caraway seeds. Similar results were observed on zinnia seeds [40].

Disinfection was used to remove the fungi from seed surface, so the results after disinfection show which fungi were located inside the seeds. This treatment favoured growth of inner inoculum, although, it should be applied carefully because

may result in the removal of a part of pathogens contaminating seed surface. Disinfection of milk thistle seeds before agar tests significantly reduced seed infestation with *A. alternata*, *Penicillium* spp. and *Verticillium* spp., whereas percentage of seeds infested with *B. cinerea* and *Cladosporium* spp. increased [31]. In the present experiment, disinfection of seeds in agar test significantly reduced percentage of several detected fungi, i.e.: *A. alternata*, *Cladosporium* spp., *Aureobasidium* sp., *Cephalosporium* sp., *E. nigrum*, *G. simplex*, *Fusarium* spp., *Penicillium* spp., *T. roseum* and *Verticillium* sp., whereas the highest percentage of seeds infested with *R. nigricans* was observed after the treatment. The abundant growth of this fungus was probably caused by the removal of competitive microorganisms. Moreover, the obtained results showed that seed disinfection might be insufficient to prevent the growth of fungi from *Mucoraceae* family in agar tests, especially if they are located inside the seeds.

Rosińska et al. [31] observed that longer incubation, i.e. 14 days instead of 10, favoured the growth of *A. alternata*, *B. cinerea*, *Fusarium* spp., *R. nigricans* and *Verticillium* spp. on milk thistle seeds. In present experiment, prolongation of incubation time also positively affected growth of *Fusarium* spp. and *Rhizopus* sp., although, had a small impact on the growth and detection of other fungi. *Rhizopus nigricans* grows fast and successfully competes with other fungi. Its competitiveness can intensify with longer incubation. From this point of view, the shorter period of seed incubation, particularly in agar tests, is recommended.

CONCLUSIONS

1. The fungi *A. alternata*, *Cladosporium* spp., *and R. nigricans* were eudominant species associated with caraway seeds.
2. The deep-freeze blotter test followed by the blotter test with an addition of mannitol performed for 10 days are recommended for further study on caraway seed health testing.

Conflict of interest: Authors declare no conflict of interest.

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INKUBACYJNE METODY WYKRYWANIA GRZYBÓW NA NASIONACH KMINKU (*CARUM CARVI* L.)

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S t r e s z c z e n i e

Wstęp: Zasiedlenie przez grzyby może istotnie wpływać na jakość nasion, jednak nie ma obecnie standardowej metody oceny zdrowotności nasion kminku (*Carum carvi* L). **Cel:** Celem prezentowanych badań było określenie optymalnej metody wykrywania grzybów zasiedlających nasiona kminku. **Metody:** Porównywano siedem metod oceny zdrowotności tych nasion: test bibułowy z przemrażaniem nasion, test bibułowy z mannitolem, test bibułowy z glikolem polietylenowym, test agarowy na pożywce glukozowo-ziemniaczanej (PDA) i zubożonej pożywce PDA (RPDA) bez odkażania nasion i test agarowy na pożywce PDA i RPDA po odkażaniu nasion. Ocenę przeprowadzono po 10 i 14 dniach inkubacji. **Wyniki:** Na nasionach stwierdzono występowanie 32 rodzajów grzybów. Najczęściej identyfikowano *Alternaria alternata*, *Cladosporium* spp. i *Rhizopus nigricans.* Wydłużenie inkubacji w największym stopniu sprzyjało wzrostowi *Fusarium* spp. i *R. nigricans*. **Wnioski:** Największe zasiedlenie nasion przez grzyby, zwłaszcza *Alternaria* spp., obserwowano w teście bibułowym z przemrażaniem nasion, a następnie w teście bibułowym z mannitolem i oba te testy można polecić do dalszych badań nad oceną zdrowotności nasion kminku.

Słowa kluczowe: *nasiona kminku, test bibułowy, test agarowy, zdrowotność nasion*