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THE QUALITY OF DILL (*ANETHUM GRAVEOLENS*) SEEDS WITH SPECIAL REFERENCE TO SEED HEALTH

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Abstract

Twelve seed lots were tested to determine quality of dill seeds produced in Poland and find the suitable method for detection and identification of fungi in/on the seeds. Germination rates were determined according to the ISTA rules. Speed and uniformity of germination characterized seed vigour. Seven methods for seed health testing were applied. The seeds showed low germination capacity, connected with high number of diseased seedlings and dead seeds. The lots differed significantly in seed vigour. *Alternaria alternata* and *Cladosporium* spp. were most frequently identified in/on the seeds. Prolongation of incubation period from 10 to 14 days had no influence on the detection of most fungi. The deep freeze blotter test, the blotter test with mannitol, and the blotter test with polyethylene glycol could be recommended for the further study on dill seeds health testing.

Key words: dill, seed germination, seed health, seed vigour

Introduction

Dill is an annual spice and medicinal plant originated from Mediterranean Region, commonly grown in Poland. The plant belongs to the Apiaceae family and its fruits are schizocarps composed of two mericarps (termed seeds), characterized by high content of essential oil. They are used for sowing purposes as well as in medicine and food industry (Dyduch 2000). The extensive use requires high quality seeds. However, fungi present on the seeds could deteriorate seeds germination (Bulajič et al. 2009, Janas et al. 1994), cause diseases of plants (Richardson 1990), and produce toxins harmful to plant and humans (Solfrizzo et al. 2005). Richardson (1990) listed the following seedborne fungi of dill: *Alternaria radicina* Meier, Drechsler & Eddy, *Cercosporidium punctum* (Lacroix) Deighton, *Chaetomium luteum*

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(Rai & Tewari) Cannon, *Dermea* sp., and *Stemphylium botryosum* Wallr. Moreover, *Alternaria alternata* (Fr.) Keissler, *Cladosporium* spp., *Epicoccum nigrum* Link., *Fusarium* spp., *Gonatobotrys simplex* Corda and *Trichothecium roseum* (Pers. ex Fr.) Link. have been frequently detected (Janas et al. 1994, Kołosowski 1994, Bralewski et al. 2005, Szopińska and Bralewski 2006, Machowicz-Stefaniak and Zalewska 2007).

Despite considerable progress in seed health testing, there are no standard methods for detecting fungi in dill seeds. Incubation blotter and agar tests are still the most common and frequently used for the detection of a great number of fungi associated with seeds. The sporulation and growth of fungi appearing on seeds have proved to be useful for identification of fungi in the blotter test. However, seed germination may be a limiting factor during evaluation. Therefore, different modifications, such as deep freezing and water restriction technique, have been proposed in order to prevent this process (Swagel et al. 1997, Machado et al. 2003, 2004, 2008, Celano et al. 2004, Falleiro et al. 2010). The fast growth of common saprotrophs, such as *Rhizopus* and *Mucor* species, seems to be the main problem in agar test (Chilvers and du Toit 2006). To overcome this difficulty surface seed disinfection has been commonly practiced (Sauer and Burroughs 1986). Moreover, the lack of sporulation can make impossible identification of some fungi in agar tests. To stimulate spore production a medium with reduced content of potato dextrose agar (PDA) has been proposed (Kohen, personal communication).

The aims of the experiment were to determine germination capacity, vigour and health of dill seeds produced in Poland and finding suitable method for detection and identification of fungi in the seeds of this species.

Materials and methods

Experiments were carried out on 12 lots of dill seed, cv. 'Amat' – three lots, cv. 'Ambrozja' – three lots, cv. 'Krezus' – two lots, and cv. 'Skaner' – four lots, produced in the years 2008 and 2009 by seed companies "Spójnia" Nochowo and "Torseed" Toruń.

Seed germination

Six replicates of 50 seeds from each lot were placed in Petri dishes containing six layers of moistened blotters and incubated in the darkness, at alternating cycle of 20 and 30°C for 16 and 8 h, respectively. Percentage of normal seedlings (germination energy and capacity), number of abnormal seedlings, both deformed and diseased, dead seeds and fresh ungerminated seeds were determined after 7 and 21 days according to the ISTA rules (International… 2006). Additionally, the total number of germinating seeds (G_{max}) was established for each lot on the basis of vigour test using statistical software SeedCalculator 2.1 (Jalink and van der Schoor 1999).

Seed vigour

To characterize seed vigour, six replicates of 50 seeds from each lot were incubated under the same conditions as described for the germination test. Radicle protrusion was scored daily for 21 days. The germination rates characterizing speed and uniformity of germination, i.e. T_1 – time to 1% of G_{max}, T_{25} – time to 25% of G_{max}, T_{75} – time to 75% of G_{max}, MGT – mean germination time and $U_{75–25}$ – time between 25% and 75% of G_{max}, were evaluated using statistical software SeedCalculator 2.1 (Jalink and van der Schoor 1999).

Evaluation of methods for detecting fungi

The following methods were applied:

Deep freeze blotter test (DFBT) – the seeds were placed in Petri dishes containing six layers of blotter moistened with distilled water. For freezing the seeds were kept for 24 h at –20°C after three days of incubation at 20°C in darkness.

Blotter test with mannitol (BT+Mn) – the seeds were placed in Petri dishes containing six layers of blotter moistened with 5 ml of mannitol at an osmotic potential –1.5 MPa (112.2 g mannitol dissolved in 1 l sterile distilled water) (Swagel et al. 1997).

Blotter test with polyethylene glycol (BT+PEG) – the seeds were placed in Petri dishes containing six layers of blotter moistened with 5 ml of polyethylene glycol solution (PEG) at an osmotic potential –1.0 MPa (284 g PEG dissolved in 1 l sterile distilled water) (Michel and Kaufmann 1973).

Agar test on potato dextrose agar (PDA) after seed disinfection (PDA+Cl) – for disinfection seeds were soaked in 1% solution of sodium hypochlorite (NaClO) for 10 min, then rinsed three times in sterile distilled water and dried with sterile blotting paper. Disinfected seeds were plated on the surface of potato dextrose agar medium (39 g of PDA per 1 l distilled water) with 100 ppm streptomycin sulphate.

Agar test on PDA without seed disinfection (PDA) – seeds were plated on the surface of potato dextrose agar medium with 100 ppm streptomycin sulphate.

Agar test on reduced PDA after seed disinfection (RPDA+Cl) – disinfected seeds (procedure of disinfection described above) were plated on the surface of reduced potato dextrose agar medium (10 g of PDA + 12 g pure agar per 1 l distilled water) with 100 ppm streptomycin sulphate.

Agar test on reduced PDA without seed disinfection (RPDA) – seeds were plated on the surface of reduced potato dextrose agar medium with 100 ppm streptomycin sulphate.

For each method and each lot 200 seeds were tested. Ten seeds per 9 cm diameter Petri dish were evenly plated on the surface of blotters or agar medium. The seeds were incubated at 20°C under alternating cycles of 12 h NUV light and 12 h darkness. The evaluation was performed twice, after 10 and 14 days of incubation. The fungi were identified on the basis of their growth and sporulation in blotter tests and on the basis of appearance of colony in agar test. In both tests stereo-

microscope and, if necessary, compound microscope were used (Machado et al. 2002, Mathur and Kongsdal 2003).

Statistical analysis

The results obtained during evaluation of methods for dill seed health testing, presented as a percentage of seeds infested with individual fungi, were compared by means of two-way analysis of variance, where lot was the first factor and method was the second. Duncan's multiple range test was applied to estimate the differences between the means at a level of $\alpha = 0.05$.

Results

Seed germination

The total number of germinating seeds ranged in tested lots from 33.0 to 91.7% and amounted to 66.9% on average (Table 1). Generally the seeds were characterized by low germination energy (21.0–69.0%; 44.2% on average) and capacity (21.0–71.0%; 45.3% on average), which was in conjunction with high number of abnormal diseased seedlings (8.0–72.0%; 23.3% on average) and dead seeds (3.0–57.0%; 21.7% on average). The lots were characterized by a low percentage of

Table 1

Germination of dill seeds

Seed lot	Total number of germinating seeds (G_{max})	Germination energy (%)	Germination capacity (%)	Abnormal seedlings		Dead seeds (%)	Fresh ungerminated seeds (%)
				deformed (%)	diseased (%)		
'Amat' I	46.3	41.0	43.0	1.0	11.0	30.0	15.0
'Amat' II	55.3	34.0	36.0	0.3	12.0	34.0	17.7
'Amat' III	89.0	69.0	71.0	1.0	18.0	4.0	6.0
'Ambrozja' I	73.0	56.0	58.0	0	15.0	11.0	16.0
'Ambrozja' II	61.7	42.0	42.0	0	20.0	38.0	0
'Ambrozja' III	70.7	45.0	46.0	0	33.0	21.0	0
'Krezus' I	89.7	25.0	25.0	0	72.0	3.0	0
'Krezus' II	91.7	42.0	44.0	0	48.0	7.0	1.0
'Skaner' I	53.7	43.0	45.0	1.0	8.0	18.0	28.0
'Skaner' II	79.7	64.0	64.0	0	21.0	9.0	6.0
'Skaner' III	59.3	48.0	48.0	0.3	10.0	27.7	14.0
'Skaner' IV	33.0	21.0	21.0	0	12.0	57.0	10.0
Average	66.9	44.2	45.3	0.3	23.3	21.7	9.5

deformed seedlings (0–1.0%; 0.3% on average) and relatively low number of fresh ungerminated seeds (0–28%; 9.5% on average).

Seed vigour

The lots differed in speed and uniformity of germination. Time to 1% of a total number of germinating seeds ranged from 0.67 to 2.06 days (1.58 days on average) (Table 2). Time to 25% of a total number of germinating seeds ranged from 1.79 to 3.18 days (2.44 days on average). The values of T_{75} parameter ranged from 2.84 to 5.12 days (3.89 days on average), mean germination time ranged from 2.59 to 4.58 days (3.47 days on average), and time from 25 to 75% of a total number of germinating seeds ranged from 0.52 to 4.23 days (1.51 days on average).

Table 2

Speed and uniformity of germination of dill seeds (days)

Seed lot	T_1	T_{25}	T_{75}	MGT	U_{75-25}
'Amat' I	2.01	2.04	3.80	3.54	1.40
'Amat' II	0.67	1.89	5.12	4.58	4.23
'Amat' III	1.89	2.57	3.93	3.66	1.36
'Ambrozja' I	1.54	2.99	4.59	3.87	1.60
'Ambrozja' II	1.11	1.79	2.91	2.59	1.13
'Ambrozja' III	0.96	2.01	3.52	2.96	1.51
'Krezus' I	1.43	2.93	4.39	3.68	1.46
'Krezus' II	1.71	3.18	4.52	3.86	1.33
'Skaner' I	2.06	3.05	4.36	3.90	1.31
'Skaner' II	1.93	2.52	3.58	3.38	1.05
'Skaner' III	1.99	2.32	2.84	2.74	0.52
'Skaner' IV	1.60	1.91	3.07	2.89	1.15
Average	1.58	2.44	3.89	3.47	1.51

T_1 – time to 1% of total number of germinating seeds, T_{25} – time to 25% of total number of germinating seeds, T_{75} – time to 75% of total number of germinating seeds, MGT – mean germination time, U_{75-25} – time between 25% and 75% of total number of germinating seeds.

Evaluation of methods for detecting fungi

Twenty nine fungal species/genera associated with the seeds were identified in tested lots (Tables 3 and 4). *Alternaria alternata, Cladosporium* spp., *Epicoccum nigrum, Fusarium* spp., *Penicillium* spp., *Rhizopus nigricans* and *Ulocladium consortiale* occurred in all examined lots (Table 3). Other fungi, i.e.: *Acremoniella atra, Alternaria radicina, A. raphani, Aspergillus* spp., *Aureobasidium* sp., *Bipolaris sorokiniana, Botrytis cinerea, Cephalosporium* sp., *Chaetomium* spp., *Colletotrichum* spp., *Curvularia* sp., *Gonatobotrys simplex, Melanospora* sp., *Mucor* spp., *Nigrospora* sp., *Papularia* sp., *Pestalotia* sp.,

Table 3

Effects of different methods on the presence of fungi frequently detected on dill seeds (means for 12 lots)

Method*	Percentage of seeds infested with						
	Alternaria alternata	*Cladosporium* spp.	*Epicoccum nigrum*	*Fusarium* spp.	*Penicillium* spp.	*Rhizopus nigricans*	*Ulocladium consortiale*
DFBT 10	59.1 c	10.9 d	4.6 f	3.6 c	0.8 b	0.3 a	1.8 fg
DFBT 14	59.6 c	11.6 de	5.1 f	5.0 d	1.0 b	0.3 a	2.0 g
BT+Mn 10	63.7 d	19.0 g	3.0 cd	0.5 ab	4.8 cde	0.3 a	1.8 fg
BT+Mn 14	66.3 d	20.7 h	4.1 de	0.7 b	6.0 e	0.3 a	2.0 g
BT+PEG 10	55.5 b	16.0 f	0.3 a	0.3 a	7.4 f	7.5 bc	1.6 ef
BT+PEG 14	56.1 b	17.4 fg	0.4 ab	0.3 a	8.8 f	7.6 bc	1.6 ef
PDA+Cl 10	30.9 a	1.7 a	0.8 ab	0.8 ab	0.1 a	1.9 a	0 a
PDA+Cl 14	31.1 a	1.8 a	0.8 ab	0.8 ab	0.1 a	2.7 a	0 a
PDA 10	56.5 b	5.8 c	2.9 c	5.4 d	3.5 c	6.8 bc	0.6 bc
PDA 14	56.6 b	6.9 c	3.1 c	6.7 d	4.1 cd	8.6 bc	0.6 cd
RPDA+Cl 10	31.3 a	3.3 b	1.2 b	0.7 ab	4.3 c	5.2 b	0.2 ab
RPDA+Cl 14	31.8 a	3.5 b	1.2 b	0.9 b	4.5 cd	8.1 bc	0.2 ab
RPDA 10	57.0 b	13.5 de	4.9 de	3.7 c	5.4 cde	10.3 c	1.0 de
RPDA 14	57.1 b	14.7 e	5.0 e	4.6 cd	5.8 de	16.8 d	1.0 de

*For each method evaluation was performed after 10 and 14 days of incubation.

DFBT – deep freeze blotter test, BT+Mn – blotter test with mannitol, BT+PEG – blotter test with polyethylene glycol, PDA+Cl – agar test on potato dextrose agar after seed disinfection, PDA – agar test on potato dextrose agar without seed disinfection, RPDA+Cl – agar test on reduced potato dextrose agar after seed disinfection, RPDA – agar test on reduced potato dextrose agar without seed disinfection.

Means in columns followed by the same letter are not significantly different at $\alpha = 0.05$ level according to Duncan's multiple range test.

Phoma sp., *Sordaria* sp., *Stemphylium botryosum*, *Trichoderma* spp., *Trichothecium roseum* and *Verticillium* spp. were found on the seeds occasionally and often only in one or few lots (Table 4).

Alternaria alternata usually infested more than 50% of seeds in most of the lots. The highest level of detecting the fungus on the seeds was noted in the blotter test with an addition of mannitol followed by the deep freeze blotter test. The blotter tests favoured also growth and identification of *A. radicina* (DFBT, BT+PEG), *Cladosporium* spp. (BT+Mn, BT+PEG), *E. nigrum* (DFBT, BT+Mn), *Penicillium* spp. (BT+PEG), *S. botryosum* (DFBT), *T. roseum* (DFBT, BT+Mn, BT+PEG) and *U. consortiale* (DFBT, BT+Mn, BT+PEG). However, agar media positively affected growth of *Aspergillus* spp. (PDA+Cl, RPDA+Cl, PDA, RPDA), *B. cinerea* (RPDA, PDA), *Chaetomium* spp. (PDA, RPDA, PDA+Cl, RPDA+Cl), *G. simplex* (RPDA), *R. nigricans* (RPDA), *Sordaria* sp. (PDA+Cl, PDA, RPDA), and *Verticillium* spp. (RPDA, PDA). For other fungi the results were not as clear. For example *Fusarium* spp. were identified in comparable percentages in the deep freeze blotter test as

Table 4

Effects of different methods on the presence of other fungi in tested lots after 14 days of incubation

Fungus	Maximum infestation in the lots (%)	Number of infested lots	Methods for which the maximum infestation was recorded (in brackets – number of lots)
Acremoniella atra	1.0–9.0	2	PDA (2)
Alternaria radicina	0.5–3.0	8	DFBT (4), BT+PEG (3), PDA (1), RPDA+Cl (1)
Alternaria raphani	0.5	1	RPDA+Cl (1)
Aspergillus spp.	0.5–2.5	7	PDA+Cl (3), RPDA+Cl (2), BT+PEG (1), PDA (1), RPDA (1)
Aureobasidium sp.	0.5–1.0	5	RPDA (3), PDA (2), PDA+Cl (1), RPDA+Cl (1)
Bipolaris sorokiniana	0.5–8.0	5	DFBT (3), RPDA (2), BT+PEG (1), RPDA+Cl (1)
Botrytis cinerea	0.5–3.0	5	RPDA (4), PDA (2), BT+Mn (1)
Cephalosporium sp.	0.5–7.5	5	DFBT (3), PDA (2), RPDA+Cl (2), RPDA (2), BT+Mn (1)
Chaetomium spp.	0.5–1.5	4	PDA (2), RPDA (2), BT+Mn (1), PDA+Cl (1), RPDA+Cl (1)
Colletotrichum sp.	0.5–1.5	3	DFBT (2), RPDA (1)
Curvularia sp.	0.5	1	BT+PEG (1)
Gonatobotrys simplex	0.5–62.0	7	RPDA (5), DFBT (1), BT+PEG (1)
Melanospora sp.	0.5–1.0	2	BT+Mn (2)
Mucor sp.	0.5–5.0	6	PDA (3), DFBT (2), RPDA (2), BT+PEG (1)
Nigrospora sp.	0.5–5.0	6	PDA (3), PDA+Cl (2), RPDA+Cl (1)
Pestalotia sp.	0.5	1	DFBT (1)
Phoma sp.	0.5–3.0	9	RPDA (4), BT+Mn (2), RPDA+Cl (2), DFBT (1)
Sordaria sp.	0.5–37.0	11	PDA+Cl (8), PDA (2), RPDA (2)
Stemphylium botryosum	2.5–19.0	8	DFBT (6), PDA (1), RPDA+Cl (1)
Trichoderma spp.	3.5–4.0	3	BT+PEG (1), PDA (1), RPDA (1)
Trichothecium roseum	0.5–54.0	9	DFBT (4), BT+Mn (2), RPDA (2), BT+PEG (1)
Verticillium spp.	0.5–18.5	4	RPDA (3), PDA (2)

For explanation see Table 3.

well as in the PDA and RPDA tests. The low number of lots infested with individual fungi also made difficult to draw conclusions.

Extension of incubation period had usually no influence on the detection of most fungi observed. Disinfection of the seeds resulted in significant decrease of seed infestation in agar tests (Table 3).

Generally, it was observed that the deep freeze blotter test, the blotter test with mannitol and the blotter test with polyethylene glycol could be applied for detecting fungi in dill seeds. It was found, using these methods, that the highest infestation with fungi characterized the lots: 'Amat' I and II, 'Ambrozja' II and III, and 'Skaner' II and IV (Tables 5–7). Regardless of the method, *A. alternata* was detected

Table 5

Effects of the deep freeze blotter test on the presence of selected fungi in tested lots after 14 days of incubation

Seed lot	Percentage of seeds infested with						
	Alternaria alternata	*Cladosporium* spp.	*Epicoccum nigrum*	*Fusarium* spp.	*Penicillium* spp.	*Rhizopus nigricans*	*Ulocladium consortiale*
'Amat' I	100.0	7.5	1.5	9.0	3.5	0	9.0
'Amat' II	100.0	6.5	19.5	6.0	0	0	1.5
'Amat' III	14.5	3.0	0.5	1.0	0.5	1.0	3.5
'Ambrozja' I	3.0	0.5	0.5	0.5	1.0	0	0.5
'Ambrozja' II	97.0	31.0	4.0	0.5	0	0	3.5
'Ambrozja' III	100.0	38.0	13.5	19.0	0	1.0	0
'Krezus' I	2.0	1.0	0.5	1.0	2.0	1.0	0
'Krezus' II	1.0	1.5	1.5	0	2.0	1.0	0.5
'Skaner' I	8.5	0	2.5	0	1.5	0	1.5
'Skaner' II	100.0	4.5	5.5	17.0	0	0	0
'Skaner' III	88.5	16.0	1.5	1.5	1.0	0	2.0
'Skaner' IV	100.0	29.0	10.0	4.0	0	0	2.0
Average	59.5	11.5	4.7	5.0	1.0	0.3	2.0

Table 6

Effects of the blotter test with mannitol on the presence of selected fungi in tested lots after 14 days of incubation

Seed lot	Percentage of seeds infested with						
	Alternaria alternata	*Cladosporium* spp.	*Epicoccum nigrum*	*Fusarium* spp.	*Penicillium* spp.	*Rhizopus nigricans*	*Ulocladium consortiale*
'Amat' I	100.0	16.0	3.0	1.0	29.0	0	6.5
'Amat' II	100.0	21.5	16.0	0	0	0	2.0
'Amat' III	15.5	7.5	0	1.5	4.0	1.5	1.0
'Ambrozja' I	2.5	2.5	0	0	18.0	0.5	0
'Ambrozja' II	93.5	56.0	0	0	1.0	0	4.5
'Ambrozja' III	100.0	70.0	8.5	2.0	0	0	4.0
'Krezus' I	25.0	1.5	1.0	0	8.0	0	0.5
'Krezus' II	35.0	4.0	3.5	0	2.0	0.5	0
'Skaner' I	37.0	1.5	2.5	0	1.0	1.0	1.0
'Skaner' II	100.0	10.0	1.0	1.0	0	0	0.5
'Skaner' III	86.5	14.0	0.5	1.0	3.0	0.5	3.0
'Skaner' IV	100.0	43.5	13.5	2.0	0	0	0.5
Average	66.3	20.7	4.1	0.7	5.5	0.3	2.0

Table 7

Effects of the blotter test with polyethylene glycol on the presence of selected fungi in tested lots after 14 days of incubation

Seed lot	Percentage of seeds infested with						
	Alternaria alternata	*Cladosporium* spp.	*Epicoccum nigrum*	*Fusarium* spp.	*Penicillium* spp.	*Rhizopus nigricans*	*Ulocladium consortiale*
'Amat' I	95.5	10.5	0.5	0	34.5	0.5	3.5
'Amat' II	96.0	16.5	0.5	0	0	0	0.5
'Amat' III	12.0	6.5	0	0	18.5	14.0	0.5
'Ambrozja' I	2.5	12.0	0	0	18.0	47.5	0
'Ambrozja' II	91.0	50.0	0	0	2.5	10.5	5.5
'Ambrozja' III	99.5	51.5	1.0	2.0	0	0.5	1.5
'Krezus' I	5.0	0.5	0	0	11.5	6.0	0
'Krezus' II	3.0	0	0	0	10.5	7.0	0
'Skaner' I	6.5	2.0	0	0	7.0	4.0	0
'Skaner' II	98.5	7.0	0	0.5	0	0.5	0
'Skaner' III	64.0	15.0	0	0	5.0	0	2.5
'Skaner' IV	100.0	37.5	2.5	1.0	0	0	0
Average	56.1	17.4	0.4	0.3	9.0	7.5	1.2

in over 90% seeds from these lots, whereas infestation of lot 'Ambrozja' I did not exceed 6% seeds. On average for all lots, *A. alternata* was detected in 56.1–66.3% seeds, *Cladosporium* spp. in 11.5–20.7% seeds, *E. nigrum* in 0.4–4.7% seeds, *Fusarium* spp. in 0.3–5.0% seeds, *Penicillium* spp. in 1.0–9.0% seeds, *R. nigricans* in 0.3–7.5% seeds and *U. consortiale* in 1.2–2.0% seeds, depending on the method.

Discussion

In the presented experiment *A. alternata* often infested more than 50% of seeds in examined lots. The high dill seed infestation with the fungus, often reaching 100%, was reported by many researchers (Janas et al. 1994, Kołosowski 1994, Szopińska and Bralewski 2006, Machowicz-Stefaniak and Zalewska 2007, Bulajič et al. 2009). Kołosowski (1994) reported that severe seed infestation with *A. alternata* interfered with proper seed health assessment, throughout limiting growth of other fungi, particularly pathogenic. From among pathogens, only *A. radicina* and *S. botryosum* were detected in tested seeds (in eight of 12 tested lots), and the level of infection was usually low. Janas et al. (1994) also observed that *A. radicina* occurred in low percentage (2.6% on average) of dill seeds. However, the results obtained by Montoya et al. (2001) showed that *A. alternata* and *S. botryosum* could reduce dill seed germination and plant vigour, without inducing specific disease symptoms in the field. In the present experiment severe seeds contamination with fungi was in conjunction with low germination energy and capacity, and high

numbers of abnormal diseased seedlings and dead seeds. Janas et al. (1994) observed that the level of seed infection was in most of the samples negatively correlated with germination capacity and plant emergence in the field. High level of dill seed infestation with *A. alternata* seems to deserve special attention from the food and pharmaceutical industries point of view, because the fungus is capable of producing several mycotoxins in infected plants, such as: alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II and III (ALTX-I, -II, -III), and tenuazonic acid (TeA). They are toxic to several animal species and can be harmful to humans due to their cytotoxic, genotoxic, and mutagenic properties (Logrieco et al. 2009).

The level of detection of particular fungi depended on the seed lot and method of seed health testing. Water restriction technique with the use of PEG and mannitol were studied in the experiment as an alternative to deep freezing to prevent seed germination in blotter test. Machado et al. (2002, 2003, 2008) investigated water restriction in the potentials range of –0.4 to –1.0 MPa using mannitol, sodium chloride and potassium chloride incorporated to agar medium and blotter. The authors observed that potentials of –0.6 to –1.0 MPa effectively inhibited radicle protrusion and had no effect on growth of fungi. Celano et al. (2004) found that blotter test with an addition of mannitol at potentials of –0.8 to –1.1 MPa prevented germination of wheat seeds, and did not influence detection of *Bipolaris sorokiniana*. Machado et al. (2004) observed that at potentials from –0.3 to –1.0 MPa, growth of some fungi was stimulated. Falleiro et al. (2010) found that growth of *A. alternata* on PDA medium was stimulated by osmotic potentials ranging from –0.35 to –1.4 MPa. Inhibition of seed germination by water restriction technique was also observed in the present study, but when PEG was used, it was connected with a decrease in dill seed infestation with *A. alternata*, *E. nigrum* and *Fusarium* spp. However, soaking of blotter with PEG solution favoured growth of *A. radicina*, *Cladosporium* spp., *Penicillium* spp. and *R. nigricans*. This phenomenon was also observed by Szopińska (2001), in relation to lettuce seeds. The author reported inhibition of growth of almost all fungi, except *Cladosporium* spp., when PEG at potentials of –0.75 to –1.5 MPa was used for water restriction in blotter test. Moreover, the lower potential the stronger restrain of the seed infestation was observed. On the contrary, it was found in present experiment that mannitol usually favoured growth of fungi, especially *A. alternata*. We observed this relation also in previous studies (Szopińska et al. 2012). Agar media stimulated growth of *Aspergillus* spp., *B. cinerea*, *Chaetomium* spp., *G. simplex*, *R. nigricans*, *Sordaria* sp., and *Verticillium* spp. However, the low number of lots infested with individual fungi made difficult to draw unambiguous conclusions. In general, oposite to Kohen (personal communication) information, we did not observe significant differences in the growth of most of the fungi on PDA and RPDA medium. Disinfection of the seeds significantly decreased seed infestation in both agar tests, showing how many of the fungi contaminated the seeds. Nevertheless, superficial infection may also be dangerous, because pathogen may spread from seeds surface into seedlings during germination process (Maude 1996).

Generally, on the basis of the obtained results it was found, that the deep freeze blotter test, the blotter test with mannitol and the blotter test with polyethylene glycol could be recommended for the further study for detecting fungi in dill seeds.

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Streszczenie

JAKOŚĆ NASION KOPRU (*ANETHUM GRAVEOLENS*)
ZE SZCZEGÓLNYM UWZGLĘDNIENIEM ICH ZDROWOTNOŚCI

Celem pracy było określenie zdolności kiełkowania, wigoru i zdrowotności nasion kopru produkowanych w Polsce oraz ustalenie najlepszej metody wykrywania i identyfikacji grzybów w nasionach tego gatunku. Testowano 12 prób nasion kopru. Określano ogólną liczbę kiełkujących nasion, energię i zdolność kiełkowania, liczbę kiełków anormalnych chorych i zdeformowanych oraz nasion zdrowych niekiełkujących i martwych. Wigor nasion badano, określając szybkość i równomierność kiełkowania. Zastosowano siedem metod oceny zdrowotności nasion: test bibułowy z przemrażaniem nasion, test bibułowy z mannitolem, test bibułowy z glikolem polietylenowym, test agarowy na pożywce dekstrozowo-ziemniaczanej (PDA) z odkażaniem nasion i bez ich odkażania oraz test agarowy ze zmniejszonym udziałem pożywki PDA z odkażaniem nasion i bez ich odkażania. Nasiona inkubowano 10 i 14 dni. Nasiona testowanych prób charakteryzowały się na ogół małą zdolnością kiełkowania, związaną z dużym udziałem siewek anormalnych chorych oraz nasion martwych. Próby różniły się znacząco szybkością i równomiernością kiełkowania. Zidentyfikowano 30 gatunków/rodzajów grzybów zasiedlających nasiona. Spośród nich najczęściej występowały: *Alternaria alternata*, *Cladosporium* spp., *Epicoccum nigrum*, *Fusarium* spp., *Gonatobotrys simplex*, *Penicillium* spp., *Rhizopus* spp., *Stemphylium botryosum* i *Ulocladium* spp. Wydłużenie okresu inkubacji nie miało wpływu na wykrywalność większości grzybów. Na podstawie otrzymanych wyników stwierdzono, że do dalszych badań nad wykrywaniem grzybów w nasionach kopru można rekomendować test bibułowy z przemrażaniem nasion, test bibułowy z mannitolem i test bibułowy z glikolem polietylenowym. Porównując zdrowotność nasion kopru za pomocą wytypowanych metod, zaobserwowano, że największym zasiedleniem przez grzyby charakteryzowały się nasiona prób: 'Amat' I i II, 'Ambrozja' II i III oraz 'Skaner' II i IV. Najczęściej wykrywano *A. alternata* i *Cladosporium* spp. Grzyb *A. alternata* zasiedlał, w zależności od metody, średnio 56,1–66,3% nasion, a grzyby rodzaju *Cladosporium* – średnio 11,5–20,7% nasion.

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