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ENHANCEMENT OF ZINNIA SEEDS BY OSMOPRIMING AND GRAPEFRUIT EXTRACT TREATMENT

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Abstract. Priming is one of the most common methods improving quality of seeds. The purpose of the research was to study effects of osmopriming combined with grapefruit extract (Biosept 33 SL) treatment on zinnia seed germination, vigour and infestation with fungi at 10 and 20°C. Two seed samples, cv. Kirke and Orys, varying in initial infestation with *Alternaria zinniae*, were treated with Biosept 33 SL solutions at concentrations of 0.05, 0.25, 0.5 and 1.0%, during and after priming in polyethylene glycol (PEG 8000). Controls were untreated seeds, seeds treated with fungicide Sarfun T 65 DS and seeds soaked in 0.05, 0.25, 0.5 and 1.0% Biosept 33 SL solution. Priming alone, as well as priming combined with grapefruit extracts treatment, significantly improved total germination and vigour of the seeds. Impact of Biosept 33 SL treatment on seed health varied among samples and was strongly influenced by initial seed infestation with fungi, applied doses of the preparation and temperature of incubation.

Key words: Biosept 33 SL, seed health, seed germination, seed vigour, priming, *Zinnia elegans*

INTRODUCTION

Osmopriming has been commonly used for improving speed, synchrony and percentage of seed germination for many years. The process permits partial seed hydration so that pregerminative metabolic activities proceed but germination is prevented [Heydecker and Coolbear 1977]. However, since the eighties, reports about deterioration of seed health after osmopriming have been presented by many authors [Biniek 1994, Dorna et al. 2001, Dorna et al. 2005b, Nascimento and West 1998, Petch 1989, Pill 1995, Tylkowska and Biniek 1996, Tylkowska and Van den Bulk 2001, Wei Yahong and Dorna 2006]. This phenomenon was observed also in osmoprimed zinnia seeds [Szopińska and Tylkowska 2009]. Zinnia (*Zinnia elegans* Jacq.), cultivated usually for cut flowers and flowerbeds, belongs to the worldwide most popular annual ornamental

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plants. In Poland *Alternaria zinniae*, *Fusarium culmorum*, *F. solani*, *F. oxysporum* and *Sclerotinia sclerotiorum* are in most cases responsible for sever damages of zinnia plants in the field [Łacicowa et al. 1979]. *Alternaria alternata*, *A. zinniae*, *Botrytis cinerea*, *Fusarium* spp. and *Penicillium* spp. were frequently detected in zinnia seeds [Łacicowa et al. 1991, Szopińska and Tylkowska 2009]. Among them, *Alternaria zinniae* has been considered the most important fungal seed-borne pathogen of zinnia, causing spotting of petals, foliage and stems as well as rotting of roots [Dimock and Osborn 1943, Łacicowa et al. 1991, Palacios et al. 1991, Richardson 1990, Wu and Yang 1992].

The antimicrobial activity of grapefruit extract has been confirmed previously by many authors [Angioni et al. 1998, Cvetnić and Vladimir-Knežević 2004, Dorna et al. 2004, Dorna et al. 2005b, Orlikowski 2001, Orlikowski and Skrzypczak 2001]. Commercial products, containing usually 33% of an extract obtained from seeds and pulp of grapefruit, are commonly used as disinfectants and preservatives in foodstuff production, natural medicine, and cosmetic industry [Cvetnić and Vladimir-Knežević 2004]. They have been also successfully used in plant protection. Biosept 33 SL is one of the preparations, recommended in organic farming in Poland.

The aim of the study was to assess the effects of osmopriming and treatment with grapefruit extract on germination, vigour and health of zinnia seeds.

MATERIALS AND METHODS

Materials. Two zinnia seeds samples, cv. Kirke (sample I) and Orys (sample II), obtained from Seed Company CNOS-PNOS in Poznań, were used in the experiment. Seeds were treated with plant origin preparation Biosept 33 SL (a.i. 33% grapefruit extract), produced by Cintamani Poland. Fungicide Sarfun T 65 DS (a.i. 20% carbendazim and 45% thiram), produced by Organika-Sarzyna, were used as an alternative chemical control. Polyethylene glycol 8000 (PEG), produced by Sigma Chemical Co., was used as priming agent.

Priming. Seeds were primed for five days at 20°C, in darkness. For the treatment, the seeds were plated on four layers of blotters moistened with 5 ml of PEG solution of an osmotic potential -1.0 MPa, placed in 9 cm diameter Petri dishes, at the rate of 50 seeds per dish. After priming, seeds were washed under running tap water for 5 min, and rinsed three times with sterile distilled water to remove PEG. Then, they were dried back at 20°C and 45% relative humidity for 24 h to equilibrium moisture content in semi-open Petri dishes.

Seed germination test. Percentage of normal seedlings (germination capacity at the final count) and abnormal seedlings, both deformed and diseased, were determined after four and ten days according to the ISTA rules [International… 2006]. Twelve replicates of 25 seeds from each treatment were placed in Petri dishes containing six layers of moistened blotters and incubated at 10 and 20°C, in darkness. Additionally, the total number of germinating seeds (G_{max}) was determined on the base of number of seeds with visible radicle counted daily.

Seed vigour test. Twelve replicates of 25 seeds were incubated under the same conditions as described in the previous test. Radicle protrusion was scored daily for ten

days. The germination rates, i.e.: T_1 – time to 1% of G_{max}, T_{50} – time to 50% of G_{max} and U_{75-25} – time between 25 and 75% of G_{max}, were evaluated using SeedCalculator 2.1 software [Jalink and Van der Schoor 1999].

Seed health test. The analysis was performed on 200 seeds from each treatment. The seeds were plated on six layers of moistened blotters placed in Petri dishes at the rate of 10 seeds per dish. They were incubated in darkness at 10 and 20°C for one day, at -20°C for 20 h and then for eight days at 10 and 20°C respectively, under 12 h alternating cycles of NUV light and darkness. After incubation, the seeds were examined for presence of fungi using a stereomicroscope and compound microscope.

Seed treatments. Germination, vigour and seed health tests were performed for the following treatments:

– non-primed untreated seeds,

– seeds soaked in 0.05, 0.25, 0.5 and 1.0% Biosept 33 SL solution for 30 min,

– seeds treated with 5 g Sarfun T 65 DS per 1 kg,

– seeds primed in PEG solution,

– seeds treated with Biosept 33 SL during priming; grapefruit extract was added in PEG solution at 0.05, 0.25, 0.5 and 1.0% concentration,

– seeds treated with Sarfun T 65 DS during priming; fungicide was added to PEG solution at concentration 5 g per 1 l PEG,

– seeds soaked in 0.05, 0.25, 0.5 and 1.0% Biosept 33 SL solution for 30 min after priming,

– seeds treated with 5 g Sarfun T 65 DS per 1 kg after priming.

Data analysis. SeedCalculator version 2.1 software [Jalink and Van der Schoor 1999] was applied to analyse germination and vigour data. All results were compared by means of variance analysis followed by the Duncan's test.

RESULTS

Treating zinnia seeds of both samples with grapefruit extract and Sarfun T 65 DS, applied during or after osmopriming, significantly increased total number of germinating seeds (G_{max}) at 10°C (tab. 1 and 2). Moreover, soaking seeds in Biosept 33 SL solution at concentrations 0.5% and 1.0%, as well as priming alone improved this parameter. At higher temperature (20°C) the treatment did not influence G_{max} of sample II. However, many times negatively affected this parameter at sample I, especially when the seeds were treated with 1.0% solution of Biosept 33 SL applied alone, 0.25, 0.5 and 1.0% grapefruit extract during priming and 0.5% extract after priming. At 10°C improvement of germination capacity was observed for seeds of sample II soaked in 0.5 and 1.0% solutions of Biosept 33 SL and for primed seeds, regardless of additional treatment. Increase in the number of normal seedlings was observed also for sample I, when the seeds were treated with Biosept 33 SL at concentrations 0.05 and 0.25% or Sarfun T 65 DS during and after priming. However, decrease of germination capacity of sample I was observed at lower temperature for seeds soaked in 0.05, 0.5 and 1.0% solution of grapefruit extract alone. Neither priming nor Biosept 33 SL and the fungicide alone improved germination capacity of zinnia seeds at 20°C. However, Biosept

Table 1. Effects of osmopriming and grapefruit extract (Biosept 33 SL) on zinnia seed germination – sample I
Tabela 1. Wpływ osmokondycjonowania i wyciągu z grejpfruta (Biosept 33 SL) na kiełkowanie nasion cynii – próba I

Treatment Traktowanie	Total number of germinating seeds Ogólna liczba nasion kiełkujących %		Germination capacity, Zdolność kiełkowania %		Abnormal deformed seedlings Kiełki anormalne zdeformowane %		Abnormal diseased seedlings Kiełki anormalne chore %	
	10°C	20°C	10°C	20°C	10°C	20°C	10°C	20°C
Control[1] – Kontrola	36.7 f	97.0 ab	7.0 b-d	71.3 b	32.3 b	0.7 fg	9.0 e	25.7 fg
Sarfun T[2]	45.7 d-f	96.3 a-c	6.0 c-e	55.7 e	34.7 ab	6.0 c-e	7.7 e	32.0 e-g
B 0.05%[3]	41.3 ef	95.7 a-c	1.7 e-g	62.0 c-e	27.0 b	1.3 e-g	19.7 cd	32.7 d-f
B 0.25%[3]	39.0 ef	97.3 a	3.3 c-f	35.3 f	16.7 c	0.3 g	34.7 b	58.7 a
B 0.5%[3]	42.3 ef	97.3 a	1.0 fg	40.7 f	26.7 b	6.0 b-d	8.0 e	46.3 b
B 1.0%[3]	48.0 de	92.7 c-e	0 g	34.7 f	29.3 b	16.0 a	7.3 e	44.3 bc
OP[4]	65.3 b	94.7 a-e	6.3 c-e	66.7 b-d	34.3 ab	2.7 d-g	20.7 cd	21.7 g
OP+Sarfun T[5]	55.0 cd	95.0 a-e	16.7 a	60.3 c-e	16.3 c	2.7 e-g	18.7 d	31.0 e-g
OP+B 0.05%[6]	53.3 cd	94.7 a-e	22.0 a	62.0 c-e	7.7 d	0 g	24.3 cd	27.7 e-g
OP+B 0.25%[6]	58.0 bc	93.0 c-e	19.3 a	59.3 de	10.7 cd	1.3 e-g	22.0 cd	29.3 e-g
OP+B 0.5%[6]	64.3 b	91.3 e	3.3 d-g	54.0 e	31.0 b	1.7 e-g	20.7 cd	34.3 c-f
OP+B 1.0%[6]	65.7 b	91.7 e	6.7 b-d	55.0 e	33.7 ab	8.3 bc	19.0 cd	28.7 e-g
OP→Sarfun T[7]	59.0 bc	95.0 a-e	19.0 a	82.0 a	28.7 b	0.7 fg	15.3 de	8.7 h
OP→B 0.05%[8]	79.0 a	95.7 a-d	22.7 a	53.7 e	9.0 cd	3.0 d-g	46.3 a	36.7 b-e
OP→B 0.25%[8]	53.0 cd	96.0 a-c	21.3 a	66.3 b-d	15.0 c	3.3 d-f	19.7 cd	24.3 fg
OP→B 0.5%[8]	78.0 a	92.0 de	14.0 ab	69.0 bc	36.0 ab	2.3 d-g	29.3 bc	22.0 g
OP→B 1.0%[8]	74.0 a	93.7 b-e	7.7 bc	40.7 f	46.7 a	12.3 ab	20.3 cd	41.7 b-d

[1] Control – untreated seeds – Kontrola – nasiona nietraktowane;
[2] seeds treated with Sarfun T 5 g kg^{-1} – nasiona traktowane preparatem Sarfun T 5 g kg^{-1};
[3] seeds soaked in 0.05, 0.25, 0.5 and 1.0% Biosept 33 SL solution for 30 min, respectively – nasiona moczone przez 30 min w 0,05, 0,25, 0,5 i 1,0% roztworze preparatu Biosept 33 SL;
[4] seeds primed in -1.0 MPa solution of PEG – nasiona kondycjonowane w -1.0 MPa roztworze PEG;
[5] seeds treated during priming with Sarfun T – 5 g l^{-1} – nasiona traktowane podczas kondycjonowania preparatem Sarfun T 5 g l^{-1};
[6] seeds treated with Biosept 33 SL at 0.05, 0.25, 0.5 and 0.1% concentration during priming, respectively – nasiona traktowane podczas kondycjonowania preparatem Biosept 33 SL o stężeniu 0,05, 0,25, 0,5 i 1,0%;
[7] seeds treated with Sarfun T after priming – 5 g kg^{-1} – nasiona traktowane po kondycjonowaniu preparatem Sarfun T – 5 g kg^{-1};
[8] seeds soaked after priming in 0.05, 0.25, 0.5 and 1.0% Biosept 33 SL solution for 30 min, respectively – nasiona moczone po kondycjonowaniu przez 30 min w 0,05, 0,25, 0,5 i 1,0% roztworze preparatu Biosept 33 SL.
Means in columns followed by the same letters are not significantly different according to the Duncan's test at the level $\alpha = 0.05$
Średnie w kolumnach oznaczone takimi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$, według testu Duncana

Table 2. Effects of osmopriming and grapefruit extract (Biosept 33 SL) on zinnia seed germination – sample II
Tabela 2. Wpływ osmokondycjonowania i wyciągu z grejpfruta (Biosept 33 SL) na kiełkowanie nasion cynii – próba II

Treatment Traktowanie	Total number of germinating seeds Ogólna liczba nasion kiełkujących %		Germination capacity Zdolność kiełkowania %		Abnormal deformed seedlings Kiełki anormalne zdeformowane %		Abnormal diseased seedlings Kiełki anormalne chore %	
	10°C	20°C	10°C	20°C	10°C	20°C	10°C	20°C
Control – Kontrola	21.0 g	83.7 a-c	1.7 gh	70.0 cd	10.0 e	1.3 f-h	1.0 g	13.3 c-f
Sarfun T	9.7 h	85.7 a-c	0 h	49.3 h	8.7 e	3.0 d-f	0.3 g	34.0 a
B 0.05%	23.7 g	89.0 a	7.0 g	51.7 gh	8.0 e	3.3 de	6.0 ef	28.7 ab
B 0.25%	19.0 g	85.3 a-c	2.0 gh	54.3 f-h	11.0 de	3.0 d-f	3.3 f	29.3 ab
B 0.5%	44.3 ef	80.3 bc	14.3 ef	56.7 f-h	17.0 cd	4.3 d	7,7 de	25.7 ab
B 1.0%	37.7 f	83.3 a-c	10.7 f	53.3 f-h	9.0 e	8.7 c	11.3 c-e	20.7 bc
OP	67.3 a-c	85.7 a-c	38.3 a	70.3 b-e	8.3 e	0.3 gh	10.0 c-e	11.3 d-h
OP+Sarfun T	58.3 cd	79.0 c	36.3 a	78.0 a-c	11.3 de	0 h	8.0 de	9.0 d-h
OP+B 0.05%	67.3 a-c	86.3 a-c	38.3 a	78.3 ab	10.0 e	0.7 gh	9.7 c-e	7.3 gh
OP+B 0.25%	53.7 de	84.0 a-c	37.3 a	80.0 a	10.7 de	2.0 e-g	9.0 de	5.0 h
OP+B 0.5%	58.7 b-d	84.0 a-c	15.7 d-f	49.3 h	30.0 ab	15.0 ab	15.7 a-c	15.0 cd
OP+B 1.0%	52.7 de	79.7 bc	11.3 f	59.0 f-h	23.3 bc	10.7 bc	8.0 de	11.0 d-g
OP→Sarfun T	59.3 b-d	86.7 ab	21.3 b-e	63.0 d-f	23.3 bc	11.3 bc	6.0 ef	7.3 f-h
OP→B 0.05%	62.0 b-d	85.7 a-c	22.7 b-d	60.0 e-g	14.0 de	12.7 bc	18.7 ab	10.7 d-h
OP→B 0.25%	68.7 ab	85.0 a-c	28.7 ab	68.3 de	12.7 de	9.3 bc	22.0 a	8.0 e-h
OP→B 0.5%	73.7 a	85.3 a-c	18.3 c-e	52.0 gh	35.3 a	19.0 a	12.3 b-d	12.7 c-f
OP→B 1.0%	74.7 a	84.7 a-c	24.3 bc	54.3 f-h	34.0 a	12.0 bc	9.3 c-e	15.0 c-e

For explanation see table 1
Objaśnienia podano pod tabelą 1

33 SL at concentrations 0.05 and 0.25% applied during the treatment improved this parameter for sample II and Sarfun T 65 DS applied after priming increase germination capacity of sample I. Other treatments usually adversely affected germination capacity at 20°C. The effect of priming and treatment with Biosept 33 SL or fungicide on the incidence of deformed and diseased seedlings varied among the samples and temperatures. In general, at 10°C considerably lower number of deformed seedlings was observed after 0.05% Biosept 33 SL or fungicide treatment during priming and 0.25% Biosept 33 SL treatment applied alone, during and after priming of sample I. The number of diseased seedlings decreased at 20°C when the seeds of sample I were treated with Sarfun T 65 DS after priming and when the seeds of sample II were primed with grapefruit extract at 0.05 and 0.25% concentrations. On the other hand, the opposite effect was also observed, when various treatments increased the number of diseased seedlings especially at 10°C, but also at 20°C.

Osmopriming significantly improved seed vigour of both samples, reduced the time needed to 1% and 50% germination of the total number of germinating seeds, expressed as T_1 and T_{50} parameters (tab. 3 and 4). In some extant the parameters were also improved, especially at 10°C, when the seeds were soaked in Biosept 33 SL preparation alone. In general, even if the seeds were additionally treated during and after priming with Biosept 33 SL or Sarfun T 65 DS, this beneficial effect was maintained at both temperatures. Treating zinnia seeds with Biosept 33 SL at concentrations 0.5 and 1.0% improved uniformity of germination ($U_{75\text{-}25}$) of sample I at 10°C, however, deterioration of this parameter was observed at 20°C after the treatment. Osmopriming positively affected $U_{75\text{-}25}$ at both temperatures, as well as treating seeds during priming with grapefruit extract at concentrations 0.25–1.0%, and after priming with Biosept 33 SL at concentration 0.5% and 1.0% or fungicide (tab. 3). In sample II, priming did not influence uniformity of germination at 10°C, but significantly improved this parameter at 20°C (tab. 4). This positive effect was also observed at 20°C for seeds primed and treated with Biosept 33 SL at lower concentrations and for seeds treated after priming with grapefruit extract at all applied doses or treated with fungicide.

The following fungi were identified in tested samples: *Acremoniella atra* (Corda) Sacc., *Acremonium strictum* W. Gams, *Alternaria alternata* (Fr.) Keissler, *A. zinniae* M.B. Ellis, *Aspergillus niger* van Tieghem, *Botrytis cinerea* Pers. ex Pers., *Cladosporium* spp., *Colletotrichum* spp., *Epicoccum purpurascens* Ehrenb. ex Schlecht., *Fusarium* spp., *Gonatobotrys simplex* Corda, *Mucor* sp., *Papulaspora* sp., *Penicillium* spp., *Rhizopus nigricans* Ehrenberg, *Trichothecium roseum* (Pers.) Link ex S.F. Gray and *Ulocladium* spp. Among them *A. alternata*, *A. zinniae*, and *Fusarium* spp. occurred most frequently (tab. 5 and 6). The samples considerable differed in seed infestation with *A. zinniae* and *Fusarium* spp. At 20°C seeds of sample I showed 70% infestation with *A. zinniae* and 13.5% infestation with *Fusarium* spp., whereas 12.5% and 97% of seeds of sample II were infested with these fungi, respectively. Osmopriming caused increase in seed infection with *A. zinniae* in sample I, at 10°C (tab. 5). However, at 20°C decrease in the percentage of seeds infected with the pathogen was observed in sample II after hydration (tab. 6). Biosept 33 SL applied alone, especially at higher doses, controlled growth of *A. alternata* and *A. zinniae* comparably to fungicide, at both temperatures in sample I (tab. 5). The preparation applied at concentrations 0.5 and

Table 3. Effects of osmopriming and treatment with fungicide and grapefruit extract (Biosept 33 SL) on zinnia seed vigour – sample I (days)
Tabela 3. Wpływ osmokondycjonowania i wyciągu z grejpfruta (Biosept 33 SL) na wigor nasion cynii – próba I (dni)

Treatment Traktowanie	T_1[1]		T_{50}[2]		U_{75-25}[3]	
	10°C	20°C	10°C	20°C	10°C	20°C
Control – Kontrola	2.71 ab	0.78 a	7.36 a	1.88 bc	2.88 ab	0.96 cd
Sarfun T	3.37 a	0.69 ab	7.45 a	1.90 ab	2.49 bc	1.51 a
B 0.05%	1.27 c	0.57 a-c	5.88 b	1.69 cd	3.43 a	1.08 bc
B 0.25%	2.13 b	0.53 a-c	6.09 b	1.52 d	2.59 bc	1.16 bc
B 0.5%	2.87 ab	0.44 b-d	5.98 b	1.88 bc	2.17 c-e	1.40 ab
B 1.0%	2.79 ab	0.29 a-c	6.01 b	2.16 a	2.28 c-e	1.76 a
OP	0.24 d	0.14 e-h	1.93 e-h	0.78 fg	1.70 d-f	0.55 e
OP+Sarfun T	0.16 d	0.18 gh	2.46 cd	1.12 e	2.65 bc	1.05 bc
OP+B 0.05%	0.15 d	0.27 d-g	2.20 c-f	0.99 ef	2.28 c-e	0.66 de
OP+B 0.25%	0.33 d	0.31 c-f	2.56 c	1.09 e	2.16 c-e	0.67 e
OP+B 0.5%	0.20 d	0.14 e-h	1.72 f-h	0.79 fg	1.68 e-g	0.63 e
OP+B 1.0%	0.64 d	0.26 c-e	1.92 d-g	0.93 ef	1.62 e-g	0.58 e
OP→Sarfun T	0.62 d	0.12 f-h	2.23 c-e	0.69 g	1.41 f-h	0.55 e
OP→B 0.05%	0.01 d	0.07 gh	1.02 i	0.64 g	1.62 e-g	0.66 de
OP→B 0.25%	0.13 d	0.04 gh	2.03 d-g	0.64 g	2.38 b-d	0.69 de
OP→B 0.5%	0.42 d	0.00 h	1.51 gh	0.40 h	1.07 gh	0.75 e
OP→B 1.0%	0.48 d	0.04 gh	1.44 h	0.60 g	0.94 h	0.68 de

[1] time to germination of 1% of the total number of germinating seeds – czas potrzebny do wykiełkowania 1% nasion z ogólnej liczby nasion kiełkujących
[2] time to germination of 50% of the total number of germinating seeds – czas potrzebny do wykiełkowania 50% nasion z ogólnej liczby nasion kiełkujących
[3] time to germination of 25 to 75% of the total number of germinating seeds – czas potrzebny do wykiełkowania od 25 do 75% nasion z ogólnej liczby nasion kiełkujących
For further explanation see table 1
Pozostałe objaśnienia podano pod tabelą 1

Table 4. Effects of osmopriming and treatment with fungicide and grapefruit extract (Biosept 33 SL) on zinnia seed vigour – sample II (days)
Tabela 4. Wpływ osmokondycjonowania i wyciągu z grejpfruta (Biosept 33 SL) na wigor nasion cynii – próba II (dni)

Treatment Traktowanie	T_1		T_{50}		U_{75-25}	
	10°C	20°C	10°C	20°C	10°C	20°C
Control – Kontrola	1.80 ab	0.27 b	6.49 a	1.17 ab	3.19 b-d	0.76 ab
Sarfun T	2.06 a	0.47 a	6.46 a	1.36 a	2.90 cd	0.82 ab
B 0.05%	0.66 c	0.13 b-d	4.59 bc	0.83 c	3.34 b-d	0.72 ab
B 0.25%	0.77 bc	0.10 cd	5.26 b	0.86 c	3.80 b-d	0.81 a
B 0.5%	1.35 cd	0.17 bc	3.89 cd	1.07 a	2.13 c-e	0.99 a
B 1.0%	1.64 c	0.25 bc	3.18 de	1.13 a	1.75 de	0.92 a
OP	0.03 e	0.00 d	2.01 fg	0.05 ef	3.19 bc	0.28 cd
OP+Sarfun T	0.14 e	0.00 d	2.41 e-g	0.29 de	3.12 ab	0.94 ab
OP+B 0.05%	0.02 e	0.00 d	1.70 gh	0.03 f	2.69 b-d	0.19 cd
OP+B 0.25%	0.27 de	0.03 b-d	3.74 d	0.48 d	3.61 ab	0.54 cd
OP+B 0.5%	0.00 e	0.03 cd	1.55 gh	0.50 d	2.48 b-d	0.58 bc
OP+B 1.0%	0.39 de	0.20 bc	3.00 d-f	0.83 c	2.55 b-d	0.58 bc
OP→Sarfun T	0.00 e	0.01 b-d	2.76 d-f	0.40 d	4.63 a	0.63 cd
OP→B 0.05%	0.00 e	0.00 d	0.65 hi	0.02 e	2.35 c-e	0.14 d
OP→B 0.25%	0.00 e	0.00 d	0.50 hi	0.11 ef	2.40 c-e	0.38 cd
OP→B 0.5%	0.00 e	0.00 d	0.17 i	0.02 f	0.82 f	0.11 d
OP→B 1.0%	0.00 e	0.00 d	0.39 i	0.05 f	1.32 ef	0.31 cd

For explanation see tables 1 and 3
Objaśnienia podano pod tabelami 1 i 3

Table 5. Effects of osmopriming and treatment with fungicide and grapefruit extract (Biosept 33 SL) on the incidence of fungi on zinnia seeds – sample I

Tabela 5. Wpływ osmokondycjonowania i wyciągu z grejpfruta (Biosept 33 SL) na zasiedlenie nasion cynii przez grzyby – próba I

Treatment Traktowanie	Infested seeds, % Zasiedlone nasiona, %						Seeds free from fungi, % Nasiona wolne od grzybów, %	
	Alternaria alternata		*Alternaria zinniae*		*Fusarium* spp.			
	10°C	20°C	10°C	20°C	10°C	20°C	10°C	20°C
Control – Kontrola	73.5 a-c	90.0 a	39.0 b	70.0 ab	19.0 gh	13.5 fg	0 f	0 f
Sarfun T	34.5 e	55.5 cd	7.5 fg	31.0 f	12.5 gh	5.0 h	47.5 a	25.5 a
B 0.05%	40.0 e	67.0 c	11.5 d-f	42.5 d-f	11.5 h	12.0 fg	20.0 d	8.0 d
B 0.25%	38.5 e	63.5 c	9.0 e-g	38.0 ef	16.0 gh	13.0 fg	44.0 a	16.0 c
B 0.5%	29.0 e	58.5 cd	5.5 g	33.5 f	30.0 f	8.0 gh	31.5 b	18.5 bc
B 1.0%	29.5 e	48.5 d	7.5 fg	31.5 f	31.5 f	12.0 fg	27.0 bc	22.0 ab
OP	79.0 ab	87.5 ab	54.5 a	72.0 a	96.5 a	97.0 a	0 f	0 f
OP+Sarfun T	59.0 d	84.5 ab	18.0 d	54.0 cd	21.0 fg	20.5 ef	14.0 e	2.5 e
OP+B 0.05%	76.0 ab	91.0 a	29.5 bc	59.0 bc	62.0 bc	72.5 b	0 f	0 f
OP+B 0.25%	72.5 bc	90.0 a	33.0 bc	64.5 a-c	62.0 bc	72.0 b	0 f	0 f
OP+B 0.5%	84.0 a	78.5 b	28.5 c	60.0 bc	53.0 c-e	61.0 bc	0 f	0 f
OP+B 1.0%	75.0 a-c	80.5 b	16.0 de	61.0 a-c	69.5 b	61.5 bc	0 f	0 f
OP→Sarfun T	36.0 e	66.0 c	14.5 de	47.0 de	3.0 i	1.0 i	45.5 a	14.0 c
OP→B 0.05%	80.0 ab	85.5 ab	26.5 c	70.0 ab	43.5 e	57.0 c	0 f	0 f
OP→B 0.25%	75.5 ab	84.5 ab	15.5 de	66.5 a-c	57.0 cd	49.0 c	0 f	0 f
OP→B 0.5%	58.0 d	79.5 b	9.5 e-g	66.5 a-c	52.0 c-e	30.0 de	0.5 f	0 f
OP→B 1.0%	63.5 cd	83.0 ab	16.0 de	65.0 a-c	47.5 de	31.5 d	1.0 f	0 f

For explanation see table 1

Objaśnienia podano pod tabelą 1

Table 6. Effects of osmopriming and treatment with fungicide and grapefruit extract (Biosept 33 SL) on the incidence of fungi on zinnia seeds – sample II

Tabela 6. Wpływ osmokondycjonowania i wyciągu z grejpfruta (Biosept 33 SL) na zasiedlenie nasion cynii przez grzyby – próba II

Treatment Traktowanie	Infested seeds, % Zasiedlone nasiona, %						Seeds free from fungi, % Nasiona wolne od grzybów, %	
	Alternaria alternata		*Alternaria zinniae*		*Fusarium* spp.			
	10°C	20°C	10°C	20°C	10°C	20°C	10°C	20°C
Control – Kontrola	67.0 ab	86.0 a	2.0 ab	12.5 a	66.0 e	97.0 ab	0 e	0 e
Sarfun T	16.0 g	49.0 e	2.0 ab	5.0 b	12.0 g	13.5 e	66.5 a	44.5 a
B 0.05%	66.5 ab	78.0 a-c	2.5 a	7.0 ab	78.5 de	95.5 bc	1.0 e	0.5 e
B 0.25%	60.0 a-c	78.0 a-c	0 b	7.0 ab	66.5 e	90.0 c	1.0 e	0 e
B 0.5%	14.5 ef	45.5 e	0.5 ab	6.0 ab	72.5 de	58.5 d	7.5 d	15.0 c
B 1.0%	20.5 fg	38.5 e	0.5 ab	4.5 b	70.5 e	56.0 d	15.5 c	18.5 bc
OP	54.5 bc	70.5 b-d	0.5 ab	4.5 b	93.0 a-c	98.5 ab	0 e	0 e
OP+Sarfun T	45.5 cd	74.5 a-d	2.0 ab	3.5 b	12.0 g	68.5 d	15.5 c	9.0 d
OP+B 0.05%	60.0 a-c	67.0 cd	1.0 ab	5.0 ab	95.0 ab	97.5 ab	0.5 e	0.5 e
OP+B 0.25%	57.5 a-c	80.0 ab	2.5 a	4.0 b	91.0 bc	98.5 ab	0 e	0 e
OP+B 0.5%	71.5 a	80.0 ab	2.0 ab	6.0 ab	93.5 a-c	100.0 a	0.5 e	0 e
OP+B 1.0%	58.0 a-c	68.5 b-d	2.5 a	4.0 b	92.5 a-c	99.0 ab	1.5 e	0 e
OP→Sarfun T	38.0 de	47.5 e	1.5 ab	6.0 ab	31.5 f	63.0 d	30.0 b	22.0 b
OP→B 0.05%	68.5 ab	80.0 ab	1.0 ab	6.5 ab	98.5 a	100.0 a	0 e	0 e
OP→B 0.25%	54.0 ab	85.0 a	1.0 ab	7.5 ab	97.5 ab	99.0 ab	0 e	0 e
OP→B 0.5%	23.5 ef	62.5 d	0 b	4.0 b	93.0 a-c	97.5 ab	2.0 de	1.0 e
OP→B 1.0%	15.0 g	46.5 e	0 b	4.5 b	85.0 cd	96.5 ab	5.5 d	0.5 e

For explanation see table 1

Objaśnienia podano pod tabelą 1

1.0% during priming and at concentrations 0.05–1.0% after hydration significantly limited growth of *A. zinniae* on heavily infested seeds of sample I, at 10°C. At higher temperature grapefruit extract also caused decrease in the percentage of the seeds infested with the pathogen, but the differences were not statistically significant (tab. 5). Generally, priming and treating seeds with grapefruit extract during or after hydration increased significantly the number of seeds infested with *Fusarium* spp., which was clearly visible in sample I, characterized by relatively low initial seed infestation (tab. 5 and 6). Even though, soaking seeds of sample II in 0.25, 0.5 and 1.0% solutions of Biosept 33 SL decreased significantly their infestation with *Fusarium* spp. at 20°C (tab. 6). Treatment with Sarfun T 65 DS effectively limited growth of these fungi, regardless of the method of application. Moreover, the fungicide treatment positively affected general seed health of both samples, expressed as a number of seeds free from fungi (tab. 5 and 6). Treatment with grapefruit extract alone, especially at 0.5 and 1.0% concentrations, also increased the number of healthy seeds whereas, this beneficial effect was not observed if the treatment was combined with priming.

DISCUSSION

Deterioration of germination capacity was noted several times in present experiment after grapefruit extract treatment, even if the seeds were treated with lower doses of preparation. It was especially visible at 20°C. However, Biosept 33 SL if applied during or after priming increased the number of normal seedlings at 10°C. Moreover, significant increase of the number of seedlings infested with fungi was observed at lower temperature after the treatment. This phenomenon allow to presume that priming, however, improved general ability of seeds to germinate in suboptimal conditions, caused deterioration of seed health. Furthermore, the application of grapefruit extract, especially during and after priming was not effective against seed-borne fungi. The increase of the number of diseased seedlings was observed also by Tylkowska and Van den Bulk [2001] in three carrot seed lots after osmopriming. The total germination after priming was comparable with control in two of three samples, however, authors reported decrease of the number of normal seedlings in all tested lots after the treatment. Szopińska and Tylkowska [2004] observed that addition of fungicide diminished the benefits of osmopriming of lettuce seeds but the same compound applied after priming reduced seed infestation, and had positive influence on seed vigour. Results of present experiment showed that fungicide applied during priming as well as applied after priming had no harmful impact on seed germination capacity and speed of germination. In both experiments the same chemicals in comparable doses were used and the time for priming of lettuce seeds was even shorter then for zinnia seeds. Presumably the thicker pericarp of zinnia seeds protected the embryo from toxic influence of fungicide. The results obtained by Van der Wolf et al. [2008] shoved, that the treatment of cabbage (*Brassica oleracea*) seeds with Biosept 33 SL at a concentrations higher than 1.0%, resulted in significant decrease in seed germination capacity. However, Szopińska et al. [2007] found that grapefruit extract even at the higher dose (Biosept 33 SL) if applied alone had no effect on germination and vigour of the seeds and controlled *A. zinniae* on zinnia

seeds as effectively as fungicide. Pięta et al. [2007] reported that dressing soybean seeds with 0.2% Biosept 33 SL effectively improved seedling emergence and significantly decreased the number of seedlings infected with *Pythium oligandrum*. The same effect was observed by Patkowska [2006] after the treatment of common bean, runner bean and pea seeds with 0.2% grapefruit extract. Dorna et al. [2004] reported that grapefruit extract at concentrations 0.2 and 0.5% controlled fungi on onion seeds as effectively as fungicide and it did not reduce seed germination capacity, seed vigour and seedling emergence. It seems to be crucial to match up properly a dose of preparation with species of treated seeds to avoid phytotoxic effects and maintain or even improve germination and vigour parameters.

The results of present experiment showed that osmopriming as well as priming combined with Sarfun T 65 DS or Biosept 33 SL treatment significantly accelerated zinnia seed germination, expressed in T_1 and T_{50} parameters at both temperatures. Positive effect of osmopriming on vigour of zinnia seeds confirmed results of previous experiments of the author [Szopińska and Tylkowska 2009]. They observed, however, that total seed infestation with fungi increased after the treatment. Moreover, before priming fungi mainly infested outer layers of seeds, but inner infection was higher after priming. The growing infestation of seeds with some fungi was also observed in present experiment after osmopriming. Infection with *A. zinniae* increased significantly in sample I at 10°C after the treatment, and infestation of seeds with *Fusarium* spp. increased in sample I at both temperatures, and in sample II at 10°C.

The antifungal activity of grapefruit extract has been confirmed previously [Angioni et al. 1998, Orlikowski 2001, Orlikowski and Skrzypczak 2001, Pięta et al. 2005]. Dorna et al. [2005a] reported that this preparation inhibited the growth of several fungi, isolated from cabbage, carrot and onion seeds, among them: *A. alternata*, *A. brassicicola*, *Botrytis aclada*, *B. cinerea*, *Fusarium avenaceum* and *F. oxysporum*. However in laboratory experiments carried out by Pięta et al. [2004] and in the field by Lenc [2007] *Alternaria* spp., especially *A. alternata* showed to be less susceptible to grapefruit extract. In present study the extract, especially applied in higher doses, reduced the number of seeds infected with *A. alternata* and *A. zinniae* as effectively as fungicide. It was clearly visible in the sample severely infested with the fungi.

Infestation of zinnia seeds of both samples with *Fusarium* spp. increased considerably after priming. Among treatments tested, only fungicide combined with osmopriming effectively reduced the number of seeds infested with those fungi. Biosept 33 SL was not effective against *Fusarium* spp., even when applied alone. Contrary to these results, Solarska [2003] reported that grapefruit extract inhibited mycelial growth of *Fusarium avenaceum* and *F. sambucinum* in laboratory experiments and effectively controlled *Fusarium* cone tip blight of hop in the field. Pięta et al. [2007] isolated fungi from soybean plants, both untreated and treated with Biosept 33 SL. The authors observed that the biological treatment considerably limited number of *Fusarium* spp. isolated from tested plant material. Similar results were obtained by Patkowska [2006, 2008] for bean and pea plants.

Although the results obtained during the experiment suggest that Biosept 33 SL combined with osmopriming can not replace fungicide in pre-sowing treatment of zinnia seeds against *Alternaria* spp. and *Fusarium* spp. because its efficacy was signifi-

cantly modified by temperature and level of seed infection, when applied alone positively affected quality of zinnia seeds.

CONCLUSIONS

1. Priming alone as well as priming combined with Biosept 33 SL treatment significantly improved total germination of zinnia seeds at 10°C.

2. Osmopriming and grapefruit extract accelerated germination of the seeds at both temperatures. The improvement in the uniformity of germination was observed at 20°C for sample I especially when the seeds were treated with grapefruit extract during priming and for sample II when seeds were treated with lower doses of preparation during priming and after priming regardless of dose of Biosept 33 SL.

3. After Biosept 33 SL treatment alone and when the seeds were treated with the highest doses of preparation during and after priming, a decrease in seed infection with *Alternaria zinniae* was noted at 10°C for higher infected sample. The same phenomenon was noted at 20°C for sample characterized by lower infection after soaking of seeds in 1.0% solution of Biosept 33 SL, and when the preparation was applied during priming at 0.25 and 1.0% concentrations and after priming in concentrations 0.5 and 1.0%.

4. Priming alone, as well as priming combined with grapefruit extract, was not effective against *Fusarium* spp. regardless of temperature and initial infection of the samples.

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POPRAWA JAKOŚCI NASION CYNII POPRZEZ OSMOKONDYCJONOWANIE I TRAKTOWANIE EKSTRAKTEM Z GREJPFRUTA

Streszczenie. Osmokondycjonowanie jest jednym z powszechnie stosowanych zabiegów poprawy jakości nasion. Celem doświadczenia było określenie wpływu osmokondycjonowania nasion cynii w połączeniu z traktowaniem ekstraktem z grejpfruta (Biosept 33 SL) na kiełkowanie, wigor i zasiedlenie nasion przez grzyby w temperaturze 10 i 20°C. Dwie próby nasion, odm. Kirke i Orys, różniące się początkowym zasiedleniem przez *Alternaria zinniae*, traktowano 0,05, 0,25, 0,5 i 1,0% roztworami preparatu Biosept 33 SL podczas kondycjonowania i po kondycjonowaniu w glikolu polietylenowym (PEG 8000). Kontrolę stanowiły nasiona nietraktowane, nasiona traktowane fungicydem Sarfun T 65 DS oraz nasiona moczone w 0,05, 0,25, 0,5 i 1,0% roztworach preparatu Biosept 33 SL. Samo kondycjonowanie, podobnie jak kondycjonowanie w połączeniu z traktowaniem ekstraktem z grejpfruta, znacząco poprawiało kiełkowanie i wigor nasion. Wpływ preparatu Biosept 33 SL na zdrowotność nasion był zróżnicowany u obu prób i znacząco uzależniony od początkowego zasiedlenia nasion przez grzyby, zastosowanej dawki preparatu i temperatury inkubacji.

Słowa kluczowe: Biosept 33 SL, zdrowotność nasion, kiełkowanie nasion, wigor nasion, kondycjonowanie, *Zinnia elegans*

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