ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 16(3) 2017, 13–25
www.acta.media.pl ISSN 1644-0692 DOI: 10.24326/asphc.2017.3.2

ORIGINAL PAPER

Accepted: 5.12.2016

EFFECTS OF OSMOPRIMING ON GERMINATION, VIGOUR AND HEALTH OF *Silybum marianum* (L.) Gaertn. SEEDS

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ABSTRACT

Priming is one of the most common methods improving seed quality. The purpose of the research was to study effects of osmopriming on milk thistle seed germination, vigour, health and location of fungi at 10 and 20°C. Seeds (achenes) of two samples (I, II) were primed in the polyethylene glycol (PEG) solutions of osmotic potentials of -1.0, -1.25 and -1.5 MPa, at 15°C for 3, 5 and 7 days. Applied variants of priming improved energy of seed germination and germination capacity at 10°C in both samples. Enhancement of these parameters was also observed at 20°C, if seeds of sample I were primed in the PEG solutions of osmotic potentials of -1.0 MPa for 7 days and -1.25 and -1.5 MPa for 5 and 7 days. Priming, regardless of time and osmotic potential of PEG, accelerated seed germination at 10 and 20°C in both samples. Fungi of genera: *Alternaria*, *Cladosporium*, *Fusarium* and *Rhizopus* were frequently identified on the seeds. After priming significantly increased seed infestation with fungi, especially in sample I. The fungi were more often detected in pericarps and seed coats than embryos. Many a time, after priming an increase in inner achene infestation was observed.

Key words: milk thistle, seed quality, location of fungi

INTRODUCTION

Milk thistle (*Silybum marianum* (L.) Gaertn.), belonging to the Asteraceae family, is one of the most important medicinal plants in Europe, cultivated commercially for fruits – achenes [Seidler-Łożykowska 2009]. Achenes of milk thistle, termed in practice as seeds, contain silymarin, complex of biologically active flavonolignans, such as silybin, silydianin, silychristin and isosilybin, exhibiting substantial antioxidative, anticancer and hematoprotective potential. Silymarin scavenges free radicles damaging cells exposed to toxins, increases glutathione responsible for detoxifying a wide range of compounds in the liver, stimulates synthesis of protein resulting in production of new cells, inhibits the synthesis of leukotrienes (mediators of inflammation) and increases the level of the antioxidant enzyme superoxide dismutase in cell cultures [Valenzuela et al. 1989, Müzes et al. 1991, Awang 1993, Morazzoni and Bombardelli 1995]. Milk thistle oil, a by-product obtained during silymarin production, is used in food industry and cosmetics [Szczucińska et al. 2003]. Considering the wide use of milk thistle in pharmacy and food industry, a high quality of seeds is essential. However, many potentially pathogenic and saprotrophic fungi, such as: *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. fumigatus* Fresen.,

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A. niger Tiegh., *Bipolaris* sp., *Sarocladium strictum* (W. Gams) Summerb. (formely *Cephalosporium acremonium* Corda), *Dreschlera* sp., *Fusarium* spp., *Penicillium* spp., *Rhizopus stolonifer* (Ehrenb.) Vuill. (formely *Rhizopus nigricans* Ehrenberg), *Stemphylium botryosum* Wallr., *Trichothecium roseum* (Pers.) Lin., *Ulocladium* spp. and *Verticillium* spp. have been identified on *S. marianum* seeds [Rosińska et al. 2013]. Important problems in milk thistle production, despite of seed health, are slow seed germination and non-uniform seed maturation, resulting from the location of seeds on plants [Sadowska and Andrzejewska 2009, ISTA 2012].

Priming is one of the most common methods improving seed quality based on controlled seed hydration to the level that permits some physiological processes of germination to occur, but prevents radicle emergence. Primed seeds germinate more rapidly and uniformly, particularly under adverse conditions [Bradford 1986, Kubala et al. 2015]. There are several techniques of priming, i.e.: hydro-, halo-, osmo- and matripriming. For osmopriming seeds are usually soaked in polyethylene glycol (PEG) solutions. This treatment is relatively safe, because the large particles of PEG are not able to penetrate seed cells [Kubala et al. 2013]. On the other hand, the side effect of osmopriming in PEG, is an increase in seed infestation with fungi [Tylkowska and van den Bulk 2001, Zhao et al. 2004]. Moreover, during priming fungi presented initially on seed surface or in outer seed parts, may penetrate inner seed tissues, leading to pre-emergence death of seedlings [Szopińska and Tylkowska 2003, 2009].

Therefore, the aim of the experiment was to study the influence of osmopriming on germination, vigour, health and location of fungi in milk thistle seeds.

MATERIALS AND METHODS

Two samples of milk thistle seeds, cv. Silma, obtained from the Institute of Natural Fibers and Medicinal Plants in Poznań, were used in the study.

Osmopriming. For priming 25 seeds were placed in 9-cm diameter Petri dishes on four layers of filter paper moistened with 5 ml of polyethylene glycol (Sigma Aldrich) solutions of osmotic potentials of -1.0, -1.25 and -1.5 MPa. Petri dishes were sealed with parafilm. Seeds were primed for 3, 5 or 7 days in darkness at 15°C. After priming, seeds were washed under tap water for 5 min and rinsed tree times in sterile distilled water to remove PEG. Subsequently, the seeds were surface dried with blotting paper and then dried back to initial moisture content in semi-open Petri dishes at 20°C and 45% RH for 48 h.

Seed germination and vigour tests. The tests were performed under the same conditions. Unprimed and primed seeds were placed in 9 cm diameter Petri dishes (20 seeds per plate) on six layers of filter paper, moistened with distilled water, and incubated at 10 and 20°C, in darkness. For each treatment 400 seeds (four replications of 100 seeds) were tested.

To evaluate seed germination the percentages of normal seedlings (energy of germination and germination capacity), abnormal seedlings, both diseased and deformed, as well as dead and fresh ungerminated seeds were determined after seven and 21 days of incubation according to the ISTA rules [ISTA 2012]. Additionally, the total number of germinating seeds (G_{max}) was calculated on the base of seed vigour test [Jalink and van der Schoor 1999].

To determine seed vigour radicle protrusion was scored daily for 21 days. The germination rates, characterising seed vigour, i.e.: T_1 – time to 1% 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 Seed Calculator 2.1. [Jalink and van der Schoor 1999].

Seed health test. To characterize seed health the percentages of seeds infested with individual fungi and seeds free of fungi were determined using deep-freeze blotter test. For each treatment 200 seeds (four replications of 50 seeds) were placed in 9 cm diameter Petri dishes on filter paper moistened with distilled water (10 seeds per plate). The seeds were incubated for two days at 10 and 20°C in darkness, next frozen at -20°C for 24 h, and then incubated for 7 days at 10 and 20°C under 12 h alternating cycles of NUV light and darkness.

To determine the location of fungi in unprimed and primed achenes for each treatment 100 achenes (five replications of 20 achene parts) were tested. The achenes were disinfected with 1% aqueous solution

of NaOCl for 10 min, then rinsed tree times with sterile distilled water and dried with sterile blotter. Next, they were soaked in sterile distilled water for 2 h and each was dissected aseptically on pericarp with seed coat and embryo. These components were placed on potato dextrose agar (PDA) medium with 100 ppm of streptomycin sulfate in 9 cm diameter Petri dishes, 5 pericarps with seed coats or 10 embryos per a dish. They were incubated at 10 and 20°C under alternating cycles of 12 h NUV light and 12 h darkness for 10 days.

In blotter test the fungi were identified on the base of their growth and sporulation, whereas in agar test on the base of colony appearance and sporulation with reference to Malone and Muskett [1964], Watanabe [2002] and Mathur and Kongsdal [2003]. The fungi were identified using stereomicroscope (magnification 50–60×) and compound microscope (magnification 400×). The names of the fungi were given according to *Index Fungorum* [www.indexfungorum.org/names/names.asp].

Data analysis. The results were evaluated by one-way analysis of variance followed by Duncan's multiple range test, at a level α = 0.05 [Kala 2002].

RESULTS

Seed germination. Sample I was characterized by a high total number of germinating seeds (G_{max}), exceeding 90%, regardless of osmotic potential of PEG solution and temperature. Moreover, an increase in G_{max} was observed in this sample at 10°C if seeds were primed in -1.0 MPa PEG solution for 5 days and -1.5 MPa PEG solution for 7 days. However, at 20°C a decrease in G_{max} was noted for seeds primed in -1.0 MPa PEG solution for 5 and 7 days and -1.25 MPa PEG solution for 7 days (tab. 1). Osmopriming of sample I seeds in -1.0 MPa PEG solution for 7 days, -1.25 MPa PEG solution for 5 and 7 days and -1.5 MPa PEG solution for 3, 5 and 7 days significantly increased energy of germination at 10°C. Moreover, all applied variants of priming improved germination capacity and decreased the percentage of dead seeds, but increased the percentage of abnormal diseased seedlings of at this temperature. Priming at 20°C, regardless of time and osmotic potential of PEG, resulted in an enhancement of energy of germination. Improvement of germination capacity was observed if sample I seeds were soaked in -1.0 MPa PEG solution for 7 days, -1.25 MPa PEG solution for 5 and 7 days and -1.5 MPa PEG solution for 5 days. On the other hand, deterioration of this parameter, associated with an increase in the percentage of abnormal diseased seedlings, was found for seeds primed in -1.25 and -1.5 MPa PEG solutions for 3 days. However, when PEG solution of osmotic potential of -1.25 MPa was applied for 5 days, the percentage of abnormal diseased seedlings was reduced. After osmopriming in -1.0 and -1.5 MPa PEG solutions for 3 and 5 days and -1.25 MPa PEG solution for 3 days a decrease in the number of dead seeds in sample I was observed.

Generally osmopriming negatively affected a total number of germinating seeds in sample II at both temperatures. However, at 10°C a significant improvement of energy of seed germination and germination capacity, associated with a decrease in the percentage of abnormal diseased seedlings was noted, especially if seeds were primed in -1.25 and -1.5 MPa solutions of PEG. Osmopriming in -1.0 and -1.25 MPa PEG solutions for 7 days, resulted in a significant increase in the percentage of dead seeds in sample II. Other variants of priming usually positively affected this parameter at 10°C. At higher temperature, priming of sample II seeds in -1.25 MPa PEG solution for 3 days and -1.5 MPa PEG solution for 5 days improved their energy of germination. Generally after priming a significant decrease in germination capacity associated with an increase in the percentage of abnormal diseased seedlings or the percentage of dead seeds was observed in this sample at 20°C (tab. 1).

Seed vigour. Priming, regardless of the osmotic potential of PEG solution and the duration of treatment, significantly accelerated seed germination in both samples at 10 and 20°C, but many a time negatively affected the uniformity of germination (U_{75-25}). Improvement of this parameter was observed only in sample II at 20°C, when seeds were primed for 3 and 5 days in -1.5 MPa PEG solution (tab. 2).

Seed health. The following fungi were identified on milk thistle seeds: *Acremoniella atra* (Corda) Sacc.,

Table 1. Effects of priming on germination of milk thistle seeds at 10 and 20°C

Treatment	Total number of germinating seeds, G_{max} (%)		Energy of germination (%)		Germination capacity (%)		Abnormal diseased seedlings (%)		Dead seeds (%)	
Sample I, 10°C										
Control*	96.8	ab**	0	a	25.0	a	8.0	a	42.3	g
-1.0/3	97.3	a-c	0.5	ab	57.8	d	29.3	c	13.0	e
-1.0/5	99.3	cd	0	a	69.3	e	17.3	b	10.3	de
-1.0/7	96.0	a	7.0	f	57.3	d	19.8	b	22.5	g
-1.25/3	97.8	a-d	0.5	ab	39.0	b	56.0	e	3.5	ab
-1.25/5	97.8	a-d	1.5	b-d	75.3	ef	16.0	b	8.3	c-e
-1.25/7	98.0	a-d	3.3	de	46.5	c	41.8	d	11.5	de
-1.5/3	97.5	a-c	4.3	ef	57.8	d	40.3	d	2.0	a
-1.5/5	98.8	b-d	1.0	bc	77.8	f	13.3	b	5.8	bc
-1.5/7	99.5	d	2.3	c-e	32.5	b	59.8	e	7.3	cd
Sample I, 20°C										
Control	99.0	d	14.3	a	48.0	b	44.8	b	7.0	b
-1.0/3	98.8	d	46.8	c	52.5	bc	44.8	b	2.8	a
-1.0/5	95.5	ab	40.0	c	57.5	bc	40.3	b	1.5	a
-1.0/7	93.0	a	45.0	c	60.5	c	34.3	ab	4.0	ab
-1.25/3	97.8	b-d	30.5	b	33.0	a	65.3	c	1.8	a
-1.25/5	99.3	d	62.5	d	73.0	d	24.5	a	2.5	ab
-1.25/7	96.5	a-c	48.0	c	63.3	cd	33.3	ab	3.5	ab
-1.5/3	98.8	d	30.3	b	32.3	a	66.0	c	1.8	a
-1.5/5	97.8	b-d	49.8	c	63.3	cd	34.5	ab	1.5	a
-1.5/7	98.8	cd	41.0	c	56.8	bc	40.8	b	3.0	ab
Sample II, 10°C										
Control	97.3	ef	2.8	a	16.8	a	62.0	g	20.0	e
-1.0/3	86.5	c	4.3	a	59.8	e	26.3	cd	12.5	d
-1.0/5	80.5	bc	14.3	b	39.5	c	31.8	ef	19.3	e
-1.0/7	75.3	b	5.0	a	32.8	b	16.0	b	50.0	g
-1.25/3	95.3	de	24.0	c	68.3	f	27.8	de	3.3	ab
-1.25/5	83.8	bc	28.0	c	61.3	e	23.0	cd	14.8	de
-1.25/7	58.8	a	15.8	b	49.0	d	12.3	a	37.8	f
-1.5/3	98.8	f	28.3	c	60.8	e	34.8	f	3.3	a
-1.5/5	92.5	d	23.5	c	64.5	ef	22.8	c	11.5	cd
-1.5/7	93.0	de	25.3	c	65.3	ef	27.5	c-e	6.8	bc
Sample II, 20°C										
Control	90.5	c	21.0	bc	41.8	f	45.5	bc	11.3	a
-1.0/3	69.3	ab	25.5	cd	32.0	de	41.5	b	26.8	b
-1.0/5	74.3	b	17.3	ab	19.8	ab	44.8	bc	32.3	b
-1.0/7	60.8	a	12.3	a	14.5	a	34.8	a	47.3	c
-1.25/3	88.3	c	35.3	e	38.5	ef	51.8	cd	9.3	a
-1.25/5	68.3	ab	26.5	cd	29.8	d	56.8	de	13.0	a
-1.25/7	67.5	ab	20.0	bc	22.8	bc	43.0	b	33.3	c
-1.5/3	85.0	c	22.5	b-d	25.8	b-d	61.8	e	12.3	a
-1.5/5	87.8	c	29.5	de	32.0	de	54.5	d	13.0	a
-1.5/7	75.3	b	26.5	cd	28.3	cd	58.0	de	13.0	a

* Control – unprimed seeds
-1.0/3, -1.0/5, -1.0/7 – seeds primed in PEG solution of osmotic potential of -1.0 MPa for 3, 5 and 7 days, respectively
-1.25/3, -1.25/5, -1.25/7 – seeds primed in PEG solution of osmotic potential of -1.25 MPa for 3, 5 and 7 days, respectively
-1.5/3, -1.5/5, -1.5/7 – seeds primed in PEG solution of osmotic potential of -1.5 MPa for 3, 5 and 7 days, respectively
** Means in columns, separately for each sample and temperature, followed by the same letter are not significantly different at $\alpha = 0.05$ level according to Duncan's test

Table 2. Effects of priming on vigour of milk thistle seeds at 10 and 20°C (days)

Treatment	T_1*		T_{75}		MGT		U_{75-25}	
Sample I, 10°C								
Control	4.57	e	6.22	f	5.85	d	0.81	a
-1.0/3	3.06	d	4.60	de	4.38	c	0.89	a-c
-1.0/5	1.86	ab	4.52	cd	3.91	b	1.23	cd
-1.0/7	2.21	ab	4.68	de	4.21	c	1.44	e
-1.25/3	2.77	d	4.76	e	4.32	c	0.85	ab
-1.25/5	1.90	a	4.36	bc	3.79	ab	1.21	d
-1.25/7	2.21	ab	4.24	bc	3.81	ab	1.10	cd
-1.5/3	2.64	cd	4.66	de	4.22	c	0.87	ab
-1.5/5	2.09	ab	4.21	b	3.72	a	1.05	b-d
-1.5/7	2.57	bc	3.94	a	3.70	a	0.76	a
Sample I, 20°C								
Control	2.65	f	3.34	e	3.22	e	0.38	a-c
-1.0/3	0.93	bc	2.39	ab	2.07	a	0.81	cd
-1.0/5	1.57	de	2.53	b-d	2.37	cd	0.55	b-d
-1.0/7	0.77	a	2.72	d	2.26	b-d	1.08	e
-1.25/3	1.68	e	2.54	a-d	2.36	cd	0.45	ab
-1.25/5	1.49	cd	2.40	a-c	2.23	a-c	0.51	b-d
-1.25/7	0.96	ab	2.38	ab	2.08	a	0.80	d
-1.5/3	1.75	de	2.24	a	2.15	ab	0.26	a
-1.5/5	1.48	de	2.70	cd	2.44	b-d	0.65	a-d
-1.5/7	1.46	cd	2.43	a-c	2.23	a-c	0.53	b-d
Sample II, 10°C								
Control	2.70	e	4.54	c	4.16	e	0.99	ab
-1.0/3	1.35	bc	4.06	b	3.44	cd	1.48	c
-1.0/5	0.68	a	3.38	a	2.78	a	1.68	c
-1.0/7	0.94	ab	4.45	bc	3.61	d	2.03	d
-1.25/3	1.70	d	3.69	a	3.24	c	1.05	ab
-1.25/5	1.14	bc	3.75	a	3.16	bc	1.49	c
-1.25/7	1.27	bc	4.49	c	3.81	cd	1.94	d
-1.5/3	1.89	d	3.56	a	3.21	bc	0.91	a
-1.5/5	1.42	c	3.31	a	2.90	ab	1.04	ab
-1.5/7	1.42	c	3.50	a	3.09	bc	1.22	bc
Sample II, 20°C								
Control	0.99	e	2.73	d	2.33	b	0.95	cd
-1.0/3	0.70	b-d	1.86	bc	1.67	a	0.78	b-d
-1.0/5	0.76	ab	1.89	bc	1.65	a	0.92	cd
-1.0/7	0.20	a	2.16	c	1.64	a	1.20	d
-1.25/3	0.55	b-d	2.13	c	1.79	a	0.95	cd
-1.25/5	0.82	cd	1.87	bc	1.70	a	0.80	bc
-1.25/7	0.45	bc	2.13	c	1.75	a	1.01	cd
-1.5/3	0.88	c	1.42	a	1.40	a	0.38	a
-1.5/5	0.74	c	1.86	b	1.63	a	0.65	ab
-1.5/7	0.68	cd	1.83	b	1.63	a	0.75	a-c

* T_1 – time to 1% of the total number of germinating seeds (G_{max}), T_{75} – time to 75% of G_{max}, MGT – mean germination time, U_{75-25} – time between 25% and 75% of G_{max}
Other explanations – see Table 1

Table 3. Effects of priming on the incidence of fungi on milk thistle seeds at 10 and 20°C (%)

Treatment	*Alternaria alternata*	*Cladosporium* spp.	*Fusarium* spp.	*Rhizopus stolonifer*	Seeds free of fungi
			Sample I, 10°C		
Control	35.5 a	2.0 a	0 a	2.5 ab	49.5 f
-1.0/3	63.0 c	9.0 b	2.0 bc	0.5 a	16.5 d
-1.0/5	49.5 b	11.5 b	0.5 ab	4.5 b	13.0 d
-1.0/7	74.5 d	40.0 c	1.0 ab	1.0 a	0 a
-1.25/3	73.5 d	17.0 b	0.5 ab	16.0 c	0 a
-1.25/5	45.5 b	12.0 b	2.0 bc	13.5 c	8.0 c
-1.25/7	63.0 c	30.0 c	1.5 a-c	1.0 ab	0 a
-1.5/3	43.0 ab	1.5 a	3.0 c	0.5 a	31.0 e
-1.5/5	60.5 c	11.5 b	2.0 bc	2.0 ab	1.5 b
-1.5/7	79.5 d	39.0 c	0 a	1.5 ab	0 a
			Sample I, 20°C		
Control	71.5 ab	10.0 b	2.0 a-c	1.0 a	13.0 c
-1.0/3	87.0 c-e	2.0 a	2.0 a-c	95.0 d	0 a
-1.0/5	89.0 d-f	30.5 c	0 a	12.0 b	0 a
-1.0/7	95.5 f	32.0 c	1.5 a-c	56.0 c	0 a
-1.25/3	76.5 a-c	10.5 b	1.0 ab	0 a	9.5 b
-1.25/5	79.0 b-d	35.0 c	1.5 ab	20.0 b	0 a
-1.25/7	94.5 f	38.0 c	1.0 ab	64.0 c	0 a
-1.5/3	66.5 a	4.5 ab	6.0 bc	12.5 b	16.0 c
-1.5/5	81.5 b-e	39.5 c	6.0 c	16.5 b	0 a
-1.5/7	89.5 ef	38.0 c	3.5 bc	55.0 c	0 a
			Sample II, 10°C		
Control	97.5 ab	4.5 a	1.0 a	0 a	-*
-1.0/3	99.0 b	9.0 ab	4.0 a	0 a	-
-1.0/5	93.5 a	14.0 bc	1.0 a	0 a	-
-1.0/7	99.0 b	18.5 cd	2.5 a	0 a	-
-1.25/3	96.5 ab	16.0 cd	2.5 a	0 a	-
-1.25/5	98.5 b	22.0 cd	2.5 a	0 a	-
-1.25/7	99.0 b	18.0 cd	2.5 a	0 a	-
-1.5/3	99.5 b	8.0 ab	4.5 a	0 a	-
-1.5/5	94.5 a	19.0 cd	5.5 a	0.5 a	-
-1.5/7	97.5 b	24.0 d	3.5 a	0 a	-
			Sample II, 20°C		
Control	99.5 d	8.5 a	9.5 ab	0 a	-
-1.0/3	94.0 ab	11.0 a	13.0 a-c	11.0 d	-
-1.0/5	96.5 a-c	8.0 a	8.5 ab	4.0 b-d	-
-1.0/7	93.0 a	11.0 a	6.5 a	2.0 a-c	-
-1.25/3	98.5 cd	5.5 a	10.5 ab	0 a	-
-1.25/5	95.0 ab	7.5 a	8.0 ab	6.0 bc	-
-1.25/7	92.0 a	10.0 a	7.0 a	2.0 a-c	-
-1.5/3	97.0 b-d	6.5 a	22.5 d	0 a	-
-1.5/5	97.0 a-c	4.0 a	19.0 cd	3.0 ab	-
-1.5/7	92.0 a	7.5 a	13.5 bc	2.0 a-c	-

For explanations – see Table 1
* not detected

Table 4. Presence of fungi before and after priming in individual parts of milk thistle achenes (seeds) at 10 and 20°C (%) – sample I

Treatment	*Alternaria alternata*		*Fusarium* spp.		*Rhizopus stolonifer*		Free of fungi parts of achenes	
Pericarp and seed coat, 10°C								
Control	2.0	a	0	a	0	a	94.0	b
-1.0/3	39.0	b-d	1.0	a	99.0	d	0	a
-1.0/5	49.0	cd	3.0	a	100.0	d	0	a
-1.0/7	40.0	b-d	3.0	a	100.0	d	0	a
-1.25/3	36.0	bc	5.0	a	91.0	c	1.0	a
-1.25/5	41.0	cd	1.0	a	99.0	d	0	a
-1.25/7	53.0	de	4.0	a	83.0	b	0	a
-1.5/3	44.0	cd	1.0	a	98.0	cd	0	a
-1.5/5	27.0	b	8.0	a	100.0	d	0	a
-1.5/7	65.0	e	5.0	a	100.0	d	0	a
Embryo, 10°C								
Control	0	a	0	a	10.0	a	90.0	d
-1.0/3	5.0	a	0	a	0	a	92.0	d
-1.0/5	3.0	a	0	a	0	a	89.0	d
-1.0/7	0	a	1.0	a	1.0	a	8.0	a
-1.25/3	2.0	a	0	a	2.0	a	55.0	b
-1.25/5	0	a	1.0	a	0	a	93.0	d
-1.25/7	2.0	a	1.0	a	0	a	88.0	d
-1.5/3	3.0	a	0	a	6.0	a	88.0	d
-1.5/5	4.0	a	0	a	20.0	a	80.0	c
-1.5/7	4.0	a	0	a	20.0	a	80.0	c
Pericarp and seed coat, 20°C								
Control	10.0	a	1.0	a	0	a	85.0	b
-1.0/3	47.0	b	2.0	ab	45.0	b	1.0	a
-1.0/5	53.0	bc	2.0	ab	99.0	c	0	a
-1.0/7	68.0	c	15.0	c	99.0	c	0	a
-1.25/3	45.0	b	3.0	ab	95.0	c	0	a
-1.25/5	51.0	bc	7.0	bc	100.0	c	0	a
-1.25/7	56.0	bc	0	a	100.0	c	0	a
-1.5/3	49.0	b	1.0	a	100.0	c	0	a
-1.5/5	51.0	bc	0	a	100.0	c	0	a
-1.5/7	47.0	b	0	a	95.0	c	0	a
Embryo, 20°C								
Control	0	a	0	a	0	a	100.0	e
-1.0/3	1.0	ab	0	a	0	a	98.0	e
-1.0/5	8.0	b-e	1.0	a	20.0	ab	45.0	b
-1.0/7	13.0	de	6.0	b	100.0	c	0	a
-1.25/3	1.0	ab	1.0	a	10.0	ab	87.0	d
-1.25/5	5.0	a-d	3.0	a	0	a	82.0	d
-1.25/7	7.0	b-e	0	a	20.0	ab	66.0	cd
-1.5/3	2.0	a-c	1.0	a	40.0	b	56.0	bc
-1.5/5	9.0	c-e	0	a	20.0	ab	7.0	a
-1.5/7	17.0	e	0	a	10.0	ab	0	a

For explanations – see Table 1

Table 5. Presence of fungi before and after priming in individual parts of milk thistle achenes (seeds) at 10 and 20°C (%) – sample II

Treatment	*Alternaria alternata*		*Fusarium* spp.		*Rhizopus stolonifer*		Free of fungi parts of achenes	
Pericarp and seed coat, 10°C								
Control	63.0	a	7.0	a	5.0	a	7.0	b
-1.0/3	93.0	b-d	14.0	a	21.0	bc	0	a
-1.0/5	87.0	b	13.0	a	8.0	ab	0	a
-1.0/7	88.0	bc	10.0	a	9.0	ab	0	a
-1.25/3	96.0	b-d	17.0	a	8.0	ab	0	a
-1.25/5	91.0	bc	8.0	a	24.0	a-c	0	a
-1.25/7	99.0	d	15.0	a	69.0	e	0	a
-1.5/3	94.0	b-d	20.0	a	35.0	cd	0	a
-1.5/5	90.0	bc	17.0	a	54.0	de	0	a
-1.5/7	96.0	bc	7.0	a	30.0	cd	0	a
Embryo, 10°C								
Control	5.0	ab	0	a	9.0	a	89.0	f
-1.0/3	31.0	c	0	a	93.0	b	0	a
-1.0/5	40.0	c	4.0	a	0	a	30.0	c
-1.0/7	40.0	c	1.0	a	0	a	23.0	c
-1.25/3	3.0	a	0	a	0	a	65.0	e
-1.25/5	15.0	b	3.0	a	8.0	a	25.0	c
-1.25/7	16.0	b	2.0	a	5.0	a	49.0	d
-1.5/3	15.0	b	0	a	0	a	77.0	e
-1.5/5	35.0	c	3.0	a	11.0	a	45.0	d
-1.5/7	12.0	b	0	a	9.0	a	6.0	ab
Pericarp and seed coat, 20°C								
Control	75.0	a	21.0	a-c	5.0	ab	-*	
-1.0/3	96.0	c	26.0	bc	10.0	a-c	-	
-1.0/5	95.0	c	23.0	a-c	0	a	-	
-1.0/7	81.0	ab	12.0	a	15.0	a-c	-	
-1.25/3	90.0	bc	27.0	bc	20.0	a-c	-	
-1.25/5	91.0	bc	18.0	a-c	21.0	bc	-	
-1.25/7	98.0	c	12.0	a	5.0	ab	-	
-1.5/3	98.0	c	30.0	c	90.0	d	-	
-1.5/5	99.0	c	11.0	a	13.0	a-c	-	
-1.5/7	99.0	c	13.0	ab	36.0	c	-	
Embryo, 20°C								
Control	20.0	a	2.0	ab	0	a	69.0	e
-1.0/3	36.0	a-c	8.0	b	20.0	ab	61.0	e
-1.0/5	31.0	ab	20.0	c	0	a	10.0	b
-1.0/7	39.0	a-c	4.0	ab	18.0	ab	0	a
-1.25/3	48.0	b-d	1.0	ab	10.0	a	44.0	d
-1.25/5	65.0	d	4.0	ab	16.0	ab	8.0	b
-1.25/7	43.0	bc	0	a	0	a	0	a
-1.5/3	37.0	a-c	5.0	ab	50.0	b	28.0	c
-1.5/5	44.0	bc	0	a	0	a	0	a
-1.5/7	53.0	cd	2.0	ab	10.0	a	0	a

For explanations – see Table 1
* not detected

Alternaria alternata, *Bipolaris sorokiniana* Shoemaker, *Bipolaris* sp., *Botrytis cinerea* Pers., *Ceratocystis* sp., *Cladosporium* spp., *Epicoccum nigrum* Link, *Fusarium* spp., *Melanospora* sp., *Melanospora simplex* (Corda) D. Hawksw., *Mortierella* sp., *Mucor* spp., *Papulaspora* sp., *Penicillium* spp., *Phoma* sp., *Rhizopus stolonifer, Sarocladium strictum*, *Sordaria* sp., *Stemphylium botryosum*, *Thamnidium* sp., *Trichothecium roseum*, *Ulocladium consortiale* (Thüm.) E.G. Simmons and *Verticillium* spp. Among them *A. alternata*, *Cladosporium* spp., *Fusarium* spp. and *R. stolonifer* prevailed in both samples (tab. 3).

In sample I priming many a time resulted in an increase in seed infestation with *A. alternata* and *Cladosporium* spp., especially at 10°C. Moreover, the percentage of seeds infested with *Fusarium* spp. increased significantly at 10°C after priming in -1.0 MPa PEG solution for 3 days, -1.25 MPa PEG solution for 5 days and -1.5 MPa PEG solution for 3 and 5 days. Soaking seeds in -1.25 MPa PEG solution for 3 and 5 days resulted also in an increase in seed infestation with *R. stolonifer* at lower temperature. Intensive growth of this fungus was observed on primed seeds at 20°C. Only seeds primed in -1.25 MPa PEG solution for 3 days were free of *R. stolonifer*. After priming generally, a decrease in the number of seeds free of fungi was observed in both temperatures in sample I.

In sample II after priming an increase in seed infestation with *Cladosporium* spp. was observed at 10°C, however the treatment did not affect growth of these fungi at 20°C. At higher temperature priming usually resulted in a small decrease in seed infestation with *A. alternata*. On the other hand *R. stolonifer* was found on primed seeds at 20°C, although it was not detected on unprimed seeds. Moreover, a significant increase in seed infestation with *Fusarium* spp. was observed after priming in -1.5 MPa PEG solution for 3 and 5 days. In sample II the seeds free of fungi were detected neither at 10 nor at 20°C (tab. 3).

Location of fungi. In sample I after priming infestation of pericarps and seed coats with *A. alternata* and *R. stolonifer* increased significantly at both temperatures (tab. 4). This phenomenon resulted in a decrease in the number of pericarps and seed coats free of fungi. Moreover, pericarps and seed coats of the achenes primed in -1.0 MPa PEG solution for 7 days and -1.25 MPa PEG solution for 5 days showed higher level of infestation with *Fusarium* spp. at 20°C compared to unprimed achenes. Infestation of embryos with fungi increased after priming mostly at 20°C. At 10°C a reduction in the number of embryos free of fungi was observed if achenes were primed in -1.0 MPa PEG solution for 7 days, -1.25 MPa PEG solution for 3 days, and -1.5 MPa PEG solution for 5 and 7 days. At higher temperature, priming in -1.0 MPa PEG solution for 5 and 7 days, -1.25 MPa PEG solution for 7 days and -1.5 MPa PEG solution for 5 and 7 days increased significantly infestation of embryos with *A. alternata*. After priming in -1.0 MPa PEG solution for 7 days and -1.5 MPa PEG solution for 3 days also an increase in embryo infestation with *R. stolonifer* was observed. Priming, except treating seeds with -1.0 MPa PEG solution, resulted in a significant reduction in the number of embryos free of fungi at 20°C.

In sample II after priming infestation of pericarps and seed coats with *A. alternata* increased significantly at both temperatures (tab. 5). Priming of achenes in -1.0 MPa PEG solution for 3 days, -1.25 MPa PEG solution for 7 days and -1.5 MPa PEG solution regardless of time increased infestation of pericarps and seed coats with *R. stolonifer* at 10°C. Priming in -1.5 MPa PEG solution for 3 and 7 days favoured growth of this fungus on pericarps and seed coats also at 20°C. After priming pericarps and seed coats free of fungi were not detected at both temperatures. Moreover, at 20°C in unprimed achenes, pericarps and seed coats free of fungi were not found either. Priming, generally resulted in an increase in embryo infestation with *A. alternata* at 10°C. Additionally, priming in -1.25 MPa PEG solution for 3, 5 and 7 days and -1.5 MPa PEG solution for 5 and 7 days favoured growth of this fungus at 20°C. Infestation of embryos with *Fusarium* spp. increased only at 20°C, when achenes were treated with -1.0 MPa PEG solution for 5 days. At 10°C a higher percentage of

embryos infestated with *R. stolonifer* than in control was noted after achene priming in -1.0 MPa PEG solution for 3 days, and at 20°C when achenes were primed in -1.5 MPa PEG solution for 3 days. Generally priming decreased the number of embryos free of fungi at both temperatures.

DISCUSSION

Beneficial effects of priming on germination of milk thistle seeds has been previously reported by Sedghi et al. [2010], Jowkar et al. [2012], Amoo Zad Khalili et al. [2013] and Parmoon et al. [2013]. However, these authors refer mostly to the effect of halopriming on seed germination and vigour in connection with tolerance to salinity stress. The experiment of Ghassemi-Golezani et al. [2016] showed that soaking milk thistle seeds for 16 h in distilled water (hydropriming) resulted in an increase of seed vigour at 20°C and improvement of seedling emergence in the field. The effect of osmopriming on germination and vigour of milk thistle seeds as well as seed health in suboptimal temperatures were not investigated until now. The obtained results varied depending on seed sample and treatment. In general osmopriming improved seed germination and vigour, especially at 10°C. On the other hand, a decrease in energy of germination and germination capacity, associated with an increase in the percentage of abnormal diseased seedlings or dead seeds, was frequently observed at 20°C. Nevertheless, all tested variants of priming significantly accelerated seed germination at both temperatures.

The results of health test as well as germination test showed, that primed seeds of milk thistle were infested with fungi to a larger extent than unprimed seeds. The deterioration of seed health during priming has been reported previously by Tylkowska and Biniek [1996] for carrot and parsley, Janas et al. [2000] for onion, carrot and cucumber, Dorna et al. [2001] for China aster, Tylkowska and van den Bulk [2001] for carrot, Szafirowska and Janas [2002] for onion, and Szopińska and Tylkowska [2009] for zinnia. Tylkowska and Biniek [1996] observed that carrot and parsley seeds primed in -0.6 MPa PEG solution at 20°C were infested with *Alternaria radicina*, *Penicillium* spp. and *Mucor* spp. to a higher degree than unprimed seeds. According to the authors, fungi located on seeds, even in a small amount, during priming may grow, spread and colonize other seeds. Nascimento and West [1998] suggest that optimum temperature and water availability during priming, as well as a presence of nutrients exuded from the seeds injured in the process of imbibition, favour the growth and spread of the fungi. Most of these fungi are saprotrophic microorganisms, however if they are present in a large amount, they may adversely affect seed quality and seedling emergence. Sadowski et al. [1996] observed that yellow lupine seed exudates favoured a growth of the fungi to a higher extent than standard potato-dextrose-agar medium. Chemical analysis revealed that seed exudates contain mostly simple carbohydrates which are able to simulate a growth of the mycelium and spore germination. Mexal and Reid [1973] clearly stated, that polyethylene glycol may be absorbed by the fungi but it is not metabolized, as many other sugars. Moreover, it is not toxic, even in the high concentrations.

According to Cwalina-Ambroziak et al. [2012] the potential pathogens of milk thistle are *A. alternata, B. cinerea*, and fungi of genera: *Fusarium*, *Phoma* and *Rhizoctonia*. In present experiment, *A. alternata* prevailed on primed as well as unprimed seeds of both samples. Moreover, priming resulted in an increase in the number of seeds infested with this fungus, especially in sample I. The percentage of seeds infested with *Fusarium* spp. was small and *B. cinerea* appeared sporadically. However, in sample II a significant increase in seed infestation with *Fusarium* spp. was observed at 20°C after priming in -1.5 MPa PEG solution for 3 and 5 days. Generally, at lower temperature priming improved germination capacity, despite of the high level of seed infestation with fungi. Moreover, at 10°C higher values of this parameter were observed than at 20°C. This phenomenon was probably connected with an abundant growth of fungi at higher temperature, which resulted in a relatively high number of abnormal diseased seedlings and

dead seeds. Even if a fungus was detected on seed at 10°C, its growth was slower and sporulation not as extensive as at 20°C. *Alternaria alternata*, which prevailed on tested seeds, may grow at temperatures ranged from 5–6.5 to 32°C, however, the optimum temperatures for its development ranged from 25 to 30°C [Lacey 1992].

The fungi more often infested pericarps and seed coats than embryos. Tylkowska and van den Bulk [2001] observed that internal parts of carrot seeds (mericarps) were more often infected by *Alternaria* spp. after priming. Szopińska and Tylkowska [2003] reported that priming of lettuce seeds (achenes) did not affect total infestation with fungi, but after the treatment *A. alternata, B. cinerea* and *Cladosporium* spp. were more often observed on inner parts of achenes, especially in pericarps and seed coats. Internal parts of primed zinnia seeds (achenes) were also more frequently infested with *A. alternata, A. zinniae, B. cinerea, Cladosporium* spp. and *Fusarium* spp. than relevant parts of unprimed seeds [Szopińska and Tylkowska 2009, Szopińska and Wojtaszek 2011]. According to Szopińska and Tylkowska [2004] it is possible that some fungi, which just contaminated surface of pericarps before priming, penetrated successively tissues of achenes (seeds) during the treatment. Referring to this phenomenon, Tylkowska and van den Bulk [2001], recommended to prime only healthy seeds or seeds with a low level of fungal infestation. An alternative may be also combining priming with fungicidal or microbial treatment, or with application of plant origin preparations.

In Poland, the milk thistle seeds are usually sawn in the first decade of April [Andrzejewska and Sadowska 2008, Kucharski 2010]. According to data published by the Institute of Meteorology and Water Management [www.imgw.pl/klimat] in the last years the average temperature in majority regions of Poland in April did not exceed 7–9°C. Despite of substantial seed infestation with fungi, applied variants of priming positively affected germination and vigour of tested seeds, especially at 10°C. Therefore, osmopriming may be recommended in Poland for improving seed germination and emergence of milk thistle.

CONCLUSIONS

1. Applied variants of seed priming significantly accelerated milk thistle seed germination at 10 and 20°C. Moreover, at lower temperature priming resulted in an improvement of seed germination in both samples.

2. Primed seeds were infested with fungi to a larger extent than unprimed seeds. The fungi more often infested pericarps and seed coats than embryos.

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