

Effect of priming with silver fir and oregano essential oils on seed germination and vigour of *Silene sendtneri*

Efekti prajminga sjemena s esencijalnim uljima jele i origana na vigor sjemena vrste *Silene sendtneri*

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ABSTRACT

In order to investigate the effects of seed priming with silver fir and oregano essential oils on certain important seedling characteristic and seed vigour of interesting endemic and horticultural species *Silene sendtneri*, the experiment was conducted based on randomized completely design with three replications. Traits such as germination rate, water content, dry weight, vigour index and photosynthetic pigments were analysed. Results revealed that the seedlings obtained with priming seeds showed increased growth, water content, vigour and photosynthetic pigment contents but decreased germination rate and dry weight compared with that obtained with non-primed seeds. Analysis of seed priming effects had demonstrated even germination rate is smaller the seedling vigour is slightly higher especially with all oregano oil treatments. We suggest that oregano oil has a potential as a priming agent for improvement of seedling synchrony, although at lower rate of germination.

Key words: essential oils, growth parameters, priming, *Silene sendtneri*

INTRODUCTION - Uvod

Seed needs to be pure, with high vigour, viability and germination rate in order to ensure high yield and productivity for efficient utilization in horticulture. Therefore, it is necessary to use different techniques for improvement of seed germination capacity, vigour and seedling establishment. Seed priming (pre-sowing seed treatments) is one of the most efficient, simple and

mostly used method (McDonald, 2000; Ghassemi-Golezani et al. 2013; Hussian et al. 2014).

The success of seed priming is under the influence of different factors and their complex interactions. Direct and indirect benefits of seed priming are: faster seedling emergence, less need for re-sowing, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield (Ghassemi-Golezani

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et al. 2013). Various pre-sowing seed techniques include osmopriming, hydropriming, halopriming, biopriming, hormonal priming, matrix priming, thermopriming and magnetopriming (Ghassemi-Golezani et al. 2013; Dalil, 2014).

In horticulture, seed priming techniques are widely used for plant protection from different abiotic and biotic stresses and for improvement of seed performances such as to reduce seedling emergence time and to achieve uniform seedling emergence and better allometrics (Ashraf & Foolad, 2005; Conrath et al. 2006).

Silene sendtneri Boiss. (Caryophyllaceae) is an endemic perennial plant of Balkan Peninsula (Jalas & Suominen, 1986) with white, decorative and scented flowers (Šilić, 1984). In Bosnia and Herzegovina, the plant inhabits mountain meadows of the alpine or subalpine vegetation zones (Šilić, 1984; Šoljan et al. 2009). As some other species of *Silene* genus with high decorative values, *S. sendtneri* can be widely used in horticulture.

Most of the seed priming includes field crops; the present study explores the effects of seed priming using silver fir and oregano essential oils on some growth parameters, photosynthetic pigments, and improvement of germination rate and germination synchrony in endemic plant, *Silene sendtneri*.

MATERIAL AND METHODS – Materijal i metode

Plant material and seed priming

Seeds of *Silene sendtneri* were collected during July, 2014. on locality Pjeskovita ravan on Mt. Ozren (43°54'191" N, 18°27'170" E; 1302 m above sea level). All seeds were kept at +4°C in the Laboratory for Plant physiology until use. Seed priming was performed using different concentrations (0, 10, 20 and 30 g/mL) of two essential oils, *Abies alba* and *Origanum vulgare* ("Liliya" Halilović d.o.o. Ilijaš, Bosnia and Herzegovina). Priming was performed by submergence of the seeds for 24 hours at +4 °C in appropriate solution, rinsing with sterile distilled water, and followed by seed drying at room temperature for 48 hours. In this way, prepared seeds were further cultivated for evaluation of priming effects on germination and seedling vigour.

Plant cultivation

Primed 160 seeds were cultivated in pots containing air-dried soil, 10 seeds per pot were cultivated, in 16 pots, for each treatment. Pots with seeds were placed in

growth chamber for 15 days under constant temperature (25°C) and humidity (70%) with 16 hours of light provided by neon light tubes. Plants were watered every two days using tap water.

Germination rate

Germination rate was calculated for each treatment according to the formula:

$$\%G = \frac{GS}{CS} \times 100$$

Where is: %G - germination percentage; GS - germinated seed; CS - cultivated seed.

Seedling length

Representative seedlings were selected for each treatment for analysis of seedling length. Seedling length was analysed using ImageJ program (ImageJ, 1.50e, Wayne Rasband National Institute of Health, USA), and using mm paper for calibration of the program. All measurements were done for 10 seedlings per treatment.

Water content

Analysis of water content was calculated according to fresh and dry mass of seedlings according to used pretreatment. Plant samples were dried after fresh mass was recorded by placing the material in the oven over night at 60°C. Percentage of water content was calculated according to the formula of Kumari et al. (2007):

$$\%WC = \frac{A - B}{A} * 100$$

Where: WC - water content; A - fresh mass of the sample; B - dry mass of the sample.

Vigour index

Seedling vigour index was calculated according to the formula of Reddy & Khan (2001):

$$SVI = \%G \times SL$$

Where: SVI - seedling vigour index; %G - germination percentage; SL - seedling length.

Table 1. Effect of pre-treatments with different essential oils (EO) on germination rate, some growth parameters and vigour of *Silene sendtneri* seedlingsTabela 1: Efekat prajminga esencijalnim uljima (EO) na stepen klijanja, neke parametre rasta i vigor *Silene sendtneri* klijanaca

Pre-treatment		G (%)	SL (cm)	WC (%)	DW (mg)	SVI
<i>Abies alba</i> EO (mg/mL)	<i>Origanum vulgare</i> EO (mg/mL)					
0	0	73,00 ^a ±1,42	1,02 ^b ±0,25	69,22 ^e ± 0,19	30,78 ^e ± 0,19	74,39 ^e
10	0	73,00 ^a ± 1,42	1,02 ^b ± 0,25	69,22 ^e ± 0,19	30,77 ^e ± 0,19	74,39 ^e
20	0	72,00 ^a ± 1,81	1,06 ^b ± 0,18	95,89 ^a ± 1,58	4,10 ^a ± 1,58	76,54 ^d
30	0	71,00 ^a ± 1,52	1,11 ^b ± 0,23	91,38 ^b ± 1,66	8,61 ^b ± 1,66	79,15 ^c
0	10	65,00 ^a ±3,03	1,34 ^a ±0,32	87,99 ^{a,b,c} ± 2,34	12,01 ^{a,b,c} ± 2,34	87,49 ^b
0	20	65,00 ^a ±3,10	1,44 ^a ±0,26	89,69 ^{a,b} ± 3,18	10,31 ^{a,b} ± 3,18	93,6 ^a
0	30	53,00 ^a ±3,83	1,47 ^a ±0,31	88,54 ^{a,b,c} ± 3,18	11,45 ^{a,b,c} ± 3,18	77,751 ^d

Data represents average values (±SD); treatments not shearing the same letter within one parameter differ significantly at $p \leq 0,05$ level according to Newman-Keuls test. Where: G - germination rate, SL - seedling length, WC - water content, DW - dry weight, SVI - seedling vigour index

Photosynthetic pigments content

Photosynthetic pigments were analysed from 80% acetone extracts, using dry plant material, by absorbance reading at 663, 646 and 440 nm according to the Arnon (1949) and quantified according to Porra et al. (1989) and Holm (1954): Chlorophyll $a = 12,25 * A_{663} - 2,55 * A_{646}$ (g/mL); Chlorophyll $b = 20,31 * A_{646} - 4,91 * A_{663}$ (g/mL); Total Chlorophylls = $17,76 * A_{646} - 7,34 * A_{663}$ (g/mL); Carotenoids = $4,69 * A_{440} - 0,267 * (A_{663} * A_{646})$ (g/mL). Final results were expressed as mg of pigment per g of dry weight.

Statistical analysis

All measurements were repeated in three replicates and values in tables represent mean value (±STDEV). Statistical analysis included analysis of variance using ANOVA and ANOVA post hoc Newman-Keuls test (Statistica 8.0 ©Copyright StatSoft, Inc. 1984-2007) at significance level of $p < 0,05$.

RESULTS AND DISCUSSION – Rezultati i diskusija

Effect of pre-treatment with different essential oils on germination rate, growth parameters and vigour of *Silene sendtneri* seedlings are presented in Table 1. As a result of priming, germination rate of *S. sendtneri* was slightly decreased when silver fir essential oil was applied and significantly decreased for oregano oil treatment. Effects of priming on germination have been investigated and showed different results depending upon species, improvements but also inhibition of germination rate have been reported (Basra et al., 2011; Afzal et al. 2012; Imran et al. 2013). This may be related to the type of seeds (plant/species) and/or can be concentration/time depended (Tzortzakakis, 2009) or it can be related to reparation of damaged proteins, RNA and DNA (Koehler et al. 1997).

Table 2. Effect of pre-treatment with different essential oils (EO) on photosynthetic pigments content of *Silene sendtneri* seedlings

Tabela 2: Efekat prajmanga esencijalnim uljima (EO) na sadržaj fotosintetskih pigmenata kljanaca *Silene sendtneri*

Pre-treatment		Chlorophyll a	Chlorophyll b	Total chlorophylls	Carotenoids
<i>Abies alba</i> EO (mg/mL)	<i>Origanum vulgare</i> EO (mg/mL)				
0	0	2,76 ^d ±0,26	0,89 ^b ±0,06	3,67 ^c ±0,22	0,78 ^d ±0,09
10	0	4,49 ^b ±1,42	3,46 ^{a,b} ±0,59	8,0 ^{a,b} ±2,03	1,16 ^{b,c,d} ±0,2 ⁷
20	0	2,47 ^d ±0,34	1,24 ^b ±0,07	3,73 ^c ±0,30	1,42 ^{a,b,c} ±0,07
30	0	2,96 ^d ±0,23	1,56 ^b ±0,50	4,55 ^{b,c} ±0,74	1,39 ^{a,b,c} ±0,17
0	10	6,03 ^{a,b} ±0,08	3,55 ^a ±0,17	9,55 ^a ±0,24	2,64 ^a ±0,10
0	20	5,92 ^b ±0,08	2,23 ^{b,c} ±0,08	8,1 ^{2b} ±0,16	1,38 ^b ±0,13
0	30	5,48 ^c ±0,30	2,49 ^{b,c} ±0,07	7,94 ^b ±0,34	1,64 ^{a,b} ±0,05

Data represents average values (±SD); treatments not shearing the same letter within one parameter differ significantly at $p \leq 0,05$ level according to Newman-Keuls test

Priming with *O. vulgare* essential oil significantly improved seedling length compared to *A. alba* treated plants and control (Table 1). Seed priming can be effectively used for an improvement of seedling growth, especially when seeds are cultivated under stress conditions (Afzal et al. 2008). Increase of seedling length was according to the rise of the *O. vulgare* essential oil concentration. Similar stimulatory effect of plant extracts was recorded also previously for some other plants (Imran et al. 2014).

Increase in water content was also recorded, especially for seedlings grown from seeds pre-treated with silver fir essential oil (Table 1). These results can implicate that seed priming using of essential oils stimulates root growth and more effective water and nutrient absorption from soil, better plant osmotic adjustment and/or the ability to prevent water waste. Namely, it is known that higher values of relative water content can increase metabolic activity, maintain and retain stomatal conductance, induce higher transpiration rate and photosynthesis in plants (Medrano et al. 2002; Matsushima et al. 2013; Pallaoro et al. 2016; Mohammadi et al. 2017).

Significant decrease of dry weight amount after priming with both essential oils was noted (Table 1) and unexpected. According to some authors, the increase in the stem length tended to be in a positive correlation with the dry weight (Matsushima et al., 2013 and references therein; Pallaoro et al. 2016; Mohammadi et al. 2017 and references therein).

Obtained different responses for seedling length and dry mass in this study may suggest that there was a disturbance in the metabolic activity of seedlings caused by some environmental factors. Significant enhancement of seedling vigour was recorded for seedlings grown from seeds treated with 20 g/mL oregano essential oil (Table 1).

According to obtained results, it is possible to presume that oregano oil may be used for better seedling synchrony of *Silene sendtneri*, although at lower rate of germination. Higher values of seedling length have been associated with superior seed vigour (Horii et al. 2007; Afzal et al. 2012; Hussian et al. 2014).

Stimulatory effects of both tested essential oils on photosynthetic pigment content of *Silene sendtneri* is recorded (Table 2), especially for chlorophyll *b*, total chlorophylls and carotenoids. Also, significant increasing of chlorophyll *a* is recorded for all treatments with oregano oil. Photosynthetic rate, biomass production, productivity capacity and plant vigour may be improved by high chlorophyll's concentrations (Sacała et al. 2016; Mohammadi et al. 2017) which are in accordance with observed results. Imbalance in water and photosynthetic pigment contents, from one hand, and dry weight, from other, suggest inadequate pigments ratio, increased stomatal openness and/or hormonal disorder.

CONCLUSION - Zaključak

Priming with silver fir and oregano essential oils on *Silene sendtneri* seedlings increased growth, water content, vigour and photosynthetic pigment contents but decreased germination rate and dry weight compared with that obtained with untreated seeds. It seems that applied seed priming demonstrated that seedling vigour is slightly higher even with smaller germination rate, especially with all oregano oil treatments. Therefore, it is possible to assume that oregano oil could be used for better seedling synchrony, although at lower rate of germination.

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SAŽETAK

Kako bi se istražili efekti prajminga sjemena s esencijalnim uljima jele i origana na određene važne karakteristike sadnica i vigor sjemena zanimljive endemične i hortikulture vrste *Silene sendtneri*, proveden je eksperiment zasnovan na potpuno slučajnom dizajnu sa tri ponavljanja. Analizirane su osobine kao što su stopa klijavosti, sadržaj vode, suha masa, indeks vigora i fotosintetski pigmenti. Rezultati su pokazali da su sadnice dobivene sa prajmingom sjemena pokazale povećani rast, sadržaj vode, vigor i sadržaj fotosintetskih pigmenta, ali i smanjenu stopu klijavosti i suhu masu u poređenju s onima dobivenim od netretiranih sjemenki. Analiza efekta prajminga sjemena pokazala je da je i pri manjoj stopi klijavosti vigor sadnica nešto veći, naročito kod svih tretmana s uljem origana. Sugeriramo da ulje origana ima potencijal kao prajming agent za poboljšanje sinhronije sadnica, mada pri nižoj stopi klijanja.