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The Influence of Stratification on Seedling Emergence and Growth of Narrow-Leaved Lavender and its Cultivars

Agnieszka Dobrowolska[1*] and Agnieszka Zawadzińska[1]

[1]West Pomeranian University of Technology in Szczecin, Department of Horticulture Papieża Pawła VI 3, 71-459 Szczecin, Poland.

Authors' contributions

This work was carried out in collaboration between all authors. Author AD designed the study, wrote the first draft of the manuscript and managed the experimental process. Author AZ managed the literature searches, performed the analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The research was conducted in the years 2009-2011. The research material included narrow-leaved lavender *Lavandula angustiolia* and its three cultivars: 'Hidcote Blue Strain', 'Hidcote Superior' and 'Rosea'. Five variants of seed stratification were used: 1. control (seeds which did not undergo stratification); 2. seeds stratified for 4 weeks; 3. seeds stratified for 6 weeks; 4 seeds stratified for 8 weeks; 5. seeds stratified for 10 weeks. The following measurements and observations were performed during the experiment: earliness of seedling emergence depending on the stratification time, biometric characteristics of the seedling - height, number of leaves, number of stems and the average root length. Seeds of narrow-leaved lavender and its cultivars, emerge more rapidly and effectively when subjected to a stratification treatment at 4°C, as compared to unstratified seeds. Regardless of the taxon, a 4-week stratification period is too short to overcome dormancy in the majority of seeds and to improve their emergence.

Keywords: Development; seedling emergence; growth; seed dormancy.

**Corresponding author: E-mail: Agnieszka.Dobrowolska@zut.edu.pl;*

1. INTRODUCTION

Lavender is a plant commonly grown in many countries as an ornamental and useful plants. The content of compounds, which is characterised by a broad biological activity, is a valuable advantage of this plant, owing to which it is used in the pharmaceutical, cosmetic and food industries and even in the area of crop protection [1,2,3,4].

Breeding lavender from seeds is still one of the basic methods of obtaining new plants. However, uneven seed germination constitutes a considerable difficulty in the production of many lavender cultivars. To accelerate germination and to make it more even, thus improving the quality of the plants obtained, the dormancy period should be eliminated. The application of pre-sowing treatments is recommended to eliminate the dormancy period of seeds. Physical methods, which have been commonly used for a long time, include, amongst other things, seed conditioning consisting of soaking and drying the seeds several times, seed stratification, vernalisation, scarification [5,6]. Breaking the dormancy by means of various substances, treatment with gibberellin, ethylene and salts, is becoming more and more common.

Sensitivity to stimuli inducing the end of dormancy appears in many plant species only after a certain period of remaining under certain environmental conditions. Seeds of many plant species from the temperate zone germinate only after stratification, i.e. several days, weeks or months of staying within a certain range of low temperatures [7,8]. Such mechanisms are considered to be a method of protection against overcoming the dormancy too early, which might occur after an accidental short-term improvement in the environmental conditions.

Inhibitors contained in seeds slow down or inhibit the germination process and they are also responsible for their dormancy. Owing to stratification, which involves keeping seeds at appropriate temperatures (usually ranging from 1 to 5^{o}C) with a simultaneous increase in the moisture level and access to oxygen, seed dormancy can be overcome. Seeds, which are stimulated for growth, germinate more quickly and evenly [9,10]. The aim of the study was to evaluate the emergence of seeds, depending on the stratification period, as well as to evaluate the quality of young plants of narrow-leaved lavender *Lavandula angustifolia* Mill. plants and its three cultivars.

2. MATERIALS AND METHODS

The research was conducted in the years 2009-2011 at the Faculty of Ornamental Plants, West Pomeranian University of Technology in Szczecin (Poland). The research material included narrow-leaved lavender *Lavandula angustfiolia* Mill. plants and its three cultivars: 'Hidcote Blue Strain', 'Hidcote Superior' and 'Rosea'. Lavender seeds were acquired from a German seed supplier - the Jelitto company. The seeds were healthy and fresh without any signs of damage or impurities. The experiment – seed stratification – was started the 8th December 2009 and 10th December 2010. Stratification was performed in accordance with the ISTA (The International Seed Testing Association). Five variants of seed stratification were used: 1. control (seeds which did not undergo stratification); 2. seeds stratified for 4 weeks; 3. seeds stratified for 6 weeks; 4 seeds stratified for 8 weeks; 5. seeds stratified for 10 weeks. The prepared and counted seeds form each of the taxa were mixed with moist sand and placed on shallow pans. This activity was repeated four times: 10,8,6 and 4 weeks before the planned sowing of the seeds. The pans with the seeds were placed in a cold room at a constant temperature of 4^{o}C, high humidity 90-95% without light and they were left

there until the sowing time. Control unstratified seeds were also prepared for sowing. Seeds after an appropriate stratification time were sown in seed trays filled with a substrate for seedling production which is manufactured by the Hollas company. Seeds were sown 14th February 2010 and 16 February 2011 on spots in rows at a distance of 3 cm, trays with seeds were placed in a heated propagator under high humidity conditions at temperatures of 22-24^{o}C until sprouting.

The emergence were calculated as the average of three replicates of 100 seeds. The mean emergence time (MET) was calculated according to Ellis and Roberts [11] and Caliskan et al. [12]:

$$MET= \Sigma(nT)/ \Sigma n$$

where:

T - is the time in days from 0 to the end of the emergence test
n - is the number of emerged seedlings on the day *t*.

The following measurements and observations were performed during the experiment: emerged seedlings, i.e. those that successfully penetrated through the cover [13], were counted, biometric characteristics of the seedling-height, number of leaves, number of stems and the average root length. The appearance of the plants was also evaluated.

The experiment was conducted as a two-factor experiment; the taxa were the first factor and the length of the stratification period constituted the other factor. The results were analysed statistically by means of two-factor variance analysis using the Statistica 10 software, the means were verified by means of Tukey's Honest Significant Difference test at a significance level of 0.05; means marked with the same letter do not differ significantly at α=0.05.

3. RESULTS AND DISCUSSION

It was shown in the experiment conducted in this study that seed stratification influences the germination dynamics of the narrow-leaved lavender and its three cultivars: 'Hidcote Blue Strain', 'Hidcote Superior' and 'Rosea' (Table 1; Figs. 1-4). In all the taxa under analysis, seeds, which were not subjected to stratification, began to emerge later (8-10 days). The emergence of these seeds was slow and uneven, as compared to stratified seeds. In the case of the 'Hidcote Superior' cultivar, only 8.6% of seeds emerged over a 4-week period and 21.4% of seeds from the 'Rosea' cultivar. The evaluation of emergence of plants from the individual taxa revealed that narrow-leaved lavender seeds (species) emerged best when stratified for a period of 6 and 10 weeks. The seeds stratified for 8 weeks initially emerged poorly and better emergence dynamics were observed only after approx. 8-10 days (Fig. 1). Similar relationships were observed for the 'Hidcote Blue Strain' cultivar. However, seeds stratified for a period of 4 weeks began to emerge 2 days later than unstratified seeds and their emergence power was higher only by 8% (Fig. 2). During the evaluation of the emergence of the 'Hidcote Superior' cultivar, it was found that seeds stratified for a period of 6-10 weeks emerged quickly (40-70%) and only single seeds emerged afterwards (Fig. 3). Stratified seeds of the 'Rosea' cultivar also emerged quite quickly and reached the maximum value during a few days.

Table 1. The effect of stratification on the mean of emergence (E) and mean emergence time (MET) - mean of years

Taxon	E (%)	MET (day)
Lavandula angustifolia	43.6 a*	31.4 ab
L. angustifolia 'Hidcote Blue Strain'	26.0 b	32.5 a
L. angustifolia 'Hidcote Superior'	46.4 a	30.2 b
L. angustifolia 'Rosea'	44.8 a	32.7 a
Treatment		
Control	14.0 d	31.3 b
4 weeks	30.0 c	32.1 ab
6 weeks	49.3 b	28.7 c
8 weeks	55.6 a	35.1 a
10 weeks	45.5 b	30.2 b

** Means marked with the same letter do not differ significantly at α=0.05*

Dormancy is overcome by means of physical and chemical methods in various plant species. Research conducted by numerous authors confirms the effectiveness of these pre-sowing treatments, which improve the seed germination ability and emergence. Coneflowers [14], e.g. purple coneflower, belong to such plants. This plant has variation in seed dormancy. Ethephon significantly increased early germination of the seeds [15,16].

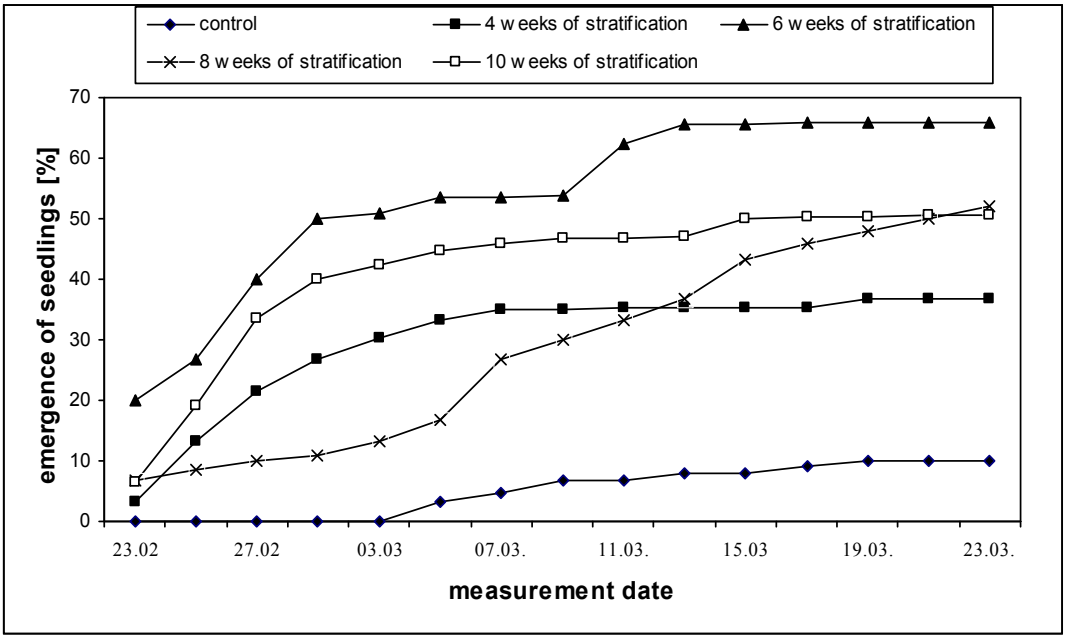

Fig. 1. Dynamics of lavender *Lavandula angustifolia* seeds emergence after stratification (mean of years)

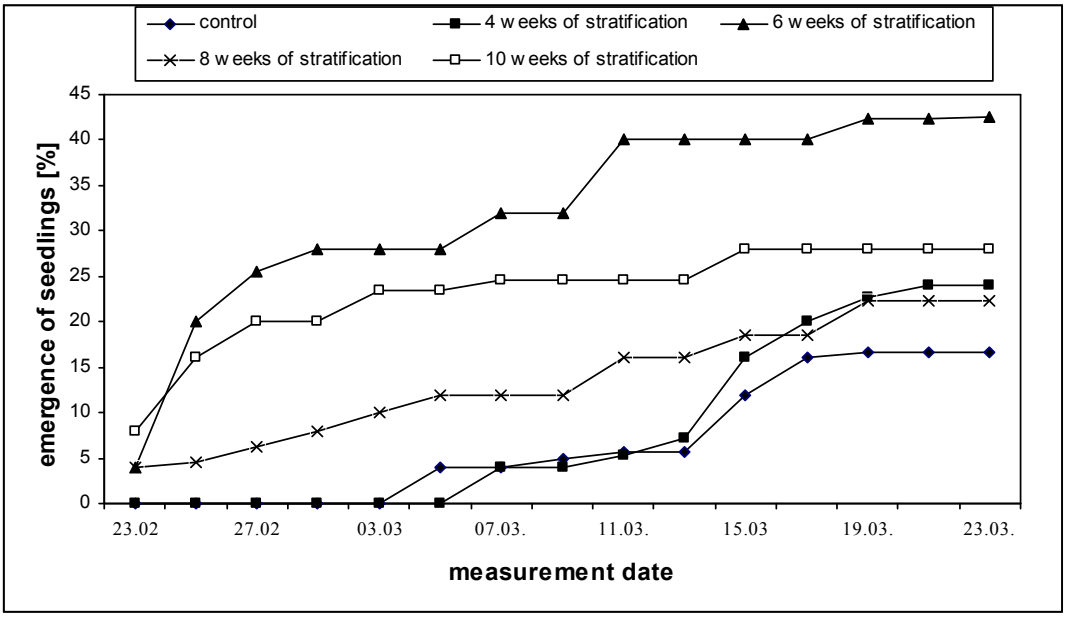

Fig. 2. Dynamics of lavender *Lavandula angustifolia* 'Hidcote Blue Strain' seeds emergence after stratification (mean of years)

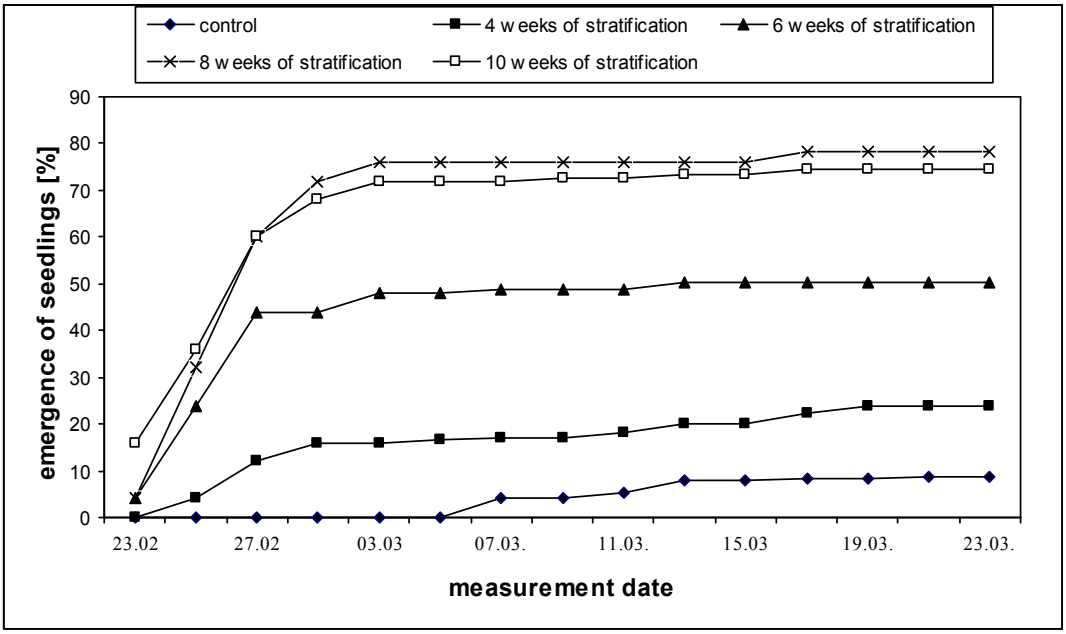

Fig. 3. Dynamics of lavender *Lavandula angustifolia* 'Hidcote Superior' seeds emergence after stratification (mean of years)

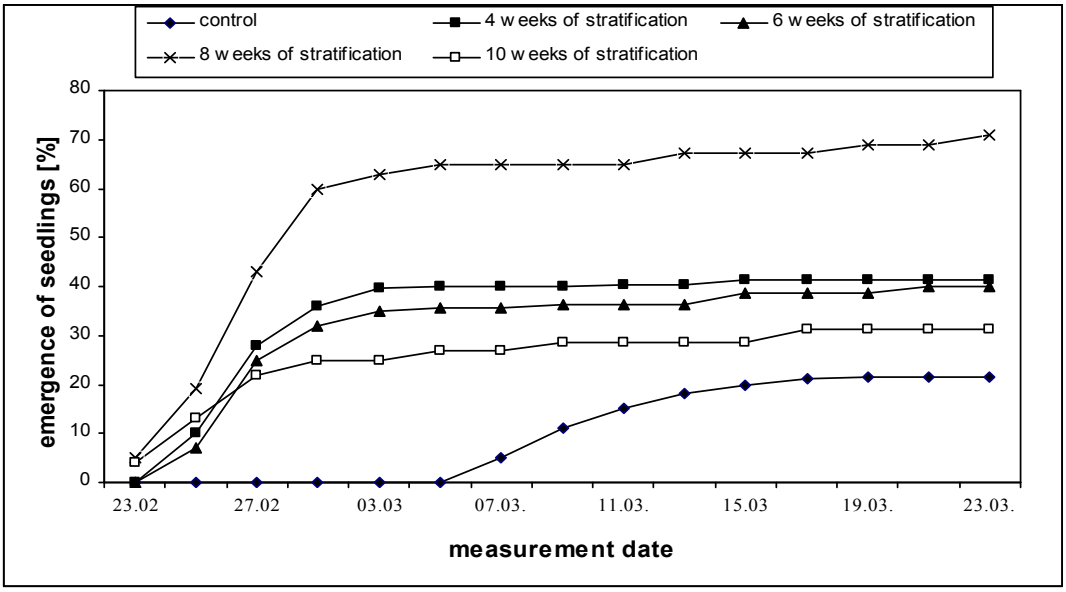

Fig. 4. Dynamics of lavender *Lavandula angustifolia* 'Rosea' seeds emergence after stratification (mean of years)

Plant dormancy is often overcome in the production of many plant species. *Lysimachia* seeds subjected to a stratification treatment germinated better than unstratified seeds [17]. *Satureja khuzistanica* seeds are in the dormant state under normal conditions and are characterised by very low germination rates. If seeds from this species are stratified, their germination ability is increased; however, a combination of several methods of breaking seed dormancy is much more advantageous. Seed stratification for a six-week period combined with soaking the seeds in GA_3 at a concentration of 500 ppm increases the seed germination ability over three times, as compared to the use of stratification only [18]. The seeds of three cucurbit species in research Mavi et al. [19] showed a wide range in the percentage of emerged seedlings in each of the four stressful sowing conditions. This was despite a similar capacity to produce high level (all above 98%) of normal seedlings in the standard germination test at 25°C.

Factors, which frequently determine the effectiveness of this treatment, include the temperature during stratification and its duration. In the research by Bratcher et al. [20], purple coneflower seeds had the highest germination rates when a four-week stratification period at 5°C was used. The extension of the stratification period for *Eucomis autumnalis* increased seed germination, the highest percentage of germinated seeds was found after 45 days of stratification [21]. Also, the conditions after sowing may be of decisive importance for germination. Both the temperature, light and humidity as well as the type of the substrate used were significant. In this study, the seeds were not subjected to preliminary germination, but were sown into a seedling substrate immediately after stratification. According to the literature on the subject, germination of plants, e.g. *Solidago*, can be as effective in substrates as on germination papers on shallow pans [22].

The 'Hidcote Superior' cultivar was characterised by the highest emergence and the largest number of emerged seedlings, while the lowest number of emerged seedlings was found for

the 'Hidcote Blue Strain' cultivar (Fig. 5). Stratification also had a significant influence on the number of emerged seedlings in the taxa under analysis. The highest percentage of emerged seedlings was found for the eight-week stratification period, the emergence rates of seeds stratified for 4 weeks were nearly twice as low. The control seeds which did not undergo stratification were characterised by the lowest emergence rates.

Not all lavender species are characterised by a long dormancy period and require stratification. The research by Delgado et al. [23] showed that *Lavandula luisieri* did not require stratification and the seed germination ability of this species was over 81%.

Various reactions of cultivars to stratification were observed; the species and the 'Hidcote Blue Strain' cultivar achieved the highest emergence rates after a six-week stratification period. Long stratification (8 and 10 weeks) proved to be the most beneficial for the 'Hidcote Superior' cultivar, while the eight-week stratification period turned out to be the most advantageous for the 'Rosea' cultivar.

Seedlings from the taxa under analysis differed in respect of their biometric features. It was shown that young plants from the *Lavandula angustifolia* species were characterised by the strongest growth; they also developed the largest number of stems and leaves (Tables 2-4). These seedlings also formed a strong root system with approx. 29 roots and an average root length of only 7.67 cm (Tables 5 and 6). The root length was the highest out of the taxa under analysis.

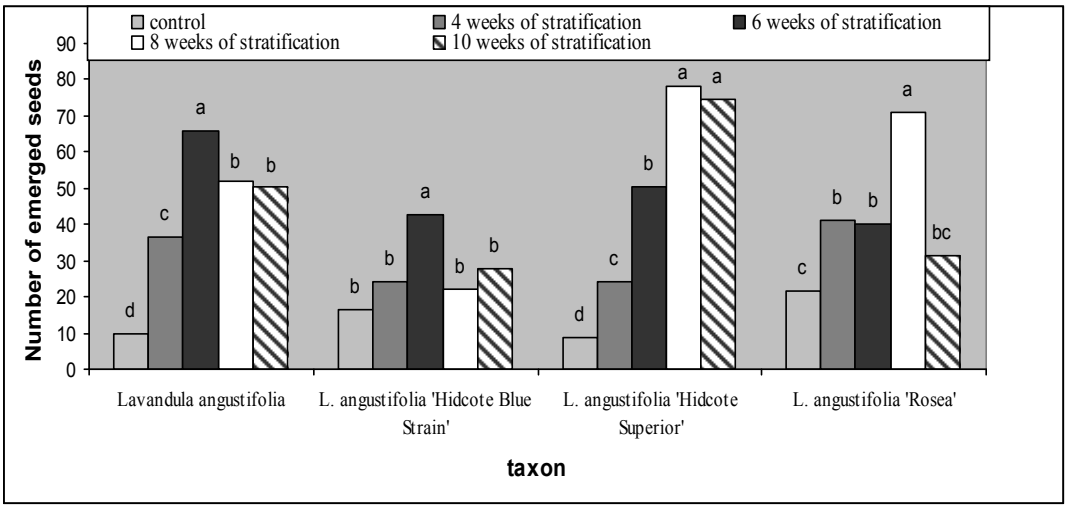

Fig. 5. Effect of stratification on the emergence of lavender seedlings and its cultivars (mean of years)

**Data marked with the same letter do not differ significantly at α=0.05*

An influence of stratification on the seedling growth was also revealed. Seedlings obtained from unstratified seeds were more delicate; they were characterised by poorer growth, they were shorter than the other variants by 2.3-5.5 cm. Seedlings obtained from seeds stratified for 10 weeks were the tallest, seedlings from seeds stratified for 6 and 8 weeks were also quite tall (Table 2).

The largest number of stems was produced by seedlings from seeds stratified for 8 weeks. Stratification for a period of at least 6 weeks also had an advantageous effect on the number of leaves. Seedlings obtained from unstratified seeds had the lowest number of stems and leaves, which results from a later onset of growth processes (Tables 4 and 5). They also formed a weaker root system with approx. 19 roots and the average root length of only 4.82 cm (Tables 5 and 6). The largest number of roots was found in plants with seeds stratified for 8 and 10 weeks; the latter also had the longest roots (Tables 5 and 6).

Table 2. Effect of stratification on the seedlings height of lavender and its cultivars (mean of years)

Seedling height [cm]					
	Taxon				
Treatment	***Lavandula angustifolia***	***L. angustifolia* 'Hidcote Blue Strain'**	***L. angustifolia* 'Hidcote Superior'**	***L. angustifolia* 'Rosea'**	**Średnia Mean**
Control	10.3 e	6.6 h	8.0 g	6.9 h	7.95 d
4 weeks	14.2 bc	7.1 gh	9.2 f	10.4 e	10.23 c
6 weeks	13.6 cd	11.1 e	14.9 b	10.5 e	12.53 b
8 weeks	14.8 b	13.2 cd	12.6 d	13.7 c	12.65 b
10 weeks	16.5 a	14.6 bc	10.1 ef	12.5 d	13.43 a
Mean	13.9 a	10.5 c	11.0 b	10.8 bc	

** Means marked with the same letter do not differ significantly at α=0.*

Table 3. Effect of stratification on the stems number of lavender seedlings and its cultivars (mean of years)

Number of stems					
	Taxon				
Treatment	***Lavandula angustifolia***	***L. angustifolia* 'Hidcote Blue Strain'**	***L. angustifolia* 'Hidcote Superior'**	***L. angustifla* 'Rosea'**	**Średnia Mean**
Control	2.0 b-e	1.3 fg	2.0 b-e	1.0 g	1.58 c
4 weeks	2.6 a	1.9 b-e	2.0 b-e	1.6 d-f	1.83 bc
6 weeks	2.4 ab	1.8 c-f	2.1 a-d	1.4 e-g	1.92 b
8 weeks	2.6 a	2.2 a-c	2.2 a-c	2.0 b-e	2.25 a
10 weeks	2.1 a-d	2.0 b-e	2.0 b-e	2.1 a-d	2.05 ab
Mean	2.34a	1.84 bc	2.06 b	1.62 c	

** Means marked with the same letter do not differ significantly at α=0.05*

Table 4. Effect of stratification on the leaves number of lavender seedlings and its cultivars (mean of years)

Number of leaves					
	Taxon				
Treatment	***Lavandula angustifolia***	***L. angustifolia* 'Hidcote Blue Strain'**	***L. angustifolia* 'Hidcote Superior'**	***L. angustifolia* 'Rosea'**	**Średnia Mean**
Control	22.3 f-h	13.5 i	21.3 gh	15.4 i	18.1 d
4 weeks	34.8 b	15.6 i	27.6 cd	18.8 h	24.2 c
6 weeks	35.4 b	30.4 cd	26.9 de	22.3 f-h	28.8 b
8 weeks	36.8 b	25.2 d-f	24.5 e-g	27.9 cd	28.6 b
10 weeks	36.4 b	42.8 a	27.6 cd	24.2 fg	32.8 a
Mean	33.1 a	25.5 b	25.6 b	21.7 c	

** Means marked with the same letter do not differ significantly at α=0.05*

Table 5. Effect of stratification on the average roots number of lavender seedlings and its cultivars (mean of years)

Average number of roots					
	Taxon				
Treatment	***Lavandula angustifolia***	***L. angustifolia* 'Hidcote Blue Strain'**	***L. angustifolia* 'Hidcote Superior'**	***L. angustifolia* 'Rosea'**	**Średnia Mean**
Control	21.2 g	17.1 h	20.8 g	16.3 h	18.9 e
4 weeks	27.4 cd	22.6 fg	26.4 de	21.3 g	24.4 d
6 weeks	29.8 b	24.8 ef	26.9 de	20.8 g	25.6 c
8 weeks	34.6 a	21.4 g	33.8 a	25.7 de	28.9 a
10 weeks	30.2 b	25.2 de	29.2 bc	26.3 de	27.7 b
Mean	28.6 a	22.2 c	27.4 b	22.1 c	

** Means marked with the same letter do not differ significantly at α=0.05*

Table 6. Effect of stratification on the average length of roots of lavender seedlings and its cultivars (mean of years)

Average length of roots [cm]					
	Taxon				
Treatment	***Lavandula angustifolia***	***L. angustifolia* 'Hidcote Blue Strain'**	***L. angustifolia* 'Hidcote Superior'**	***L. angustifolia* 'Rosea'**	**Średnia Mean**
Control	6.81 cd	3.93 gh	5.31 ef	3.24 h	4.82 c
4 weeks	7.63 a-c	5.42 ef	6.71 cd	5.53 ef	6.32 b
6 weeks	7.15 b-d	7.12 b-d	7.44 bc	4.66 fg	6.59 b
8 weeks	8.12 ab	4.86 fg	6.23 de	6.82 cd	6.51 b
10 weeks	8.72 a	8.13 ab	6.81 cd	6.72 cd	7.60 a
Mean	7.69 a	5.89 c	6.50 b	5.39 d	

** Means marked with the same letter do not differ significantly at α=0.05*

4. CONCLUSION

1. Seeds of narrow-leaved lavender and its cultivars - 'Hidcote Blue Strain', 'Hidcote Superior' and 'Rosea', germinate more rapidly and effectively when subjected to a stratification treatment at 4^{o}C, as compared to unstratified seeds.
2. The length of the stratification period depends on the taxon. The species and the 'Hidcote Blue Strain' cultivar germinated the best when a 6-week stratification period was used, while the 'Hidcote Superior' and 'Rosea' cultivars preferred an 8-week stratification period.
3. Regardless of the taxon, a 4-week stratification period is too short to overcome dormancy in the majority of seeds and to improve their germination.
4. Plants obtained from seeds stratified for a period of at least 6 weeks are better developed as compared to unstratified ones, their above-ground parts are better formed and their root systems are well-developed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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