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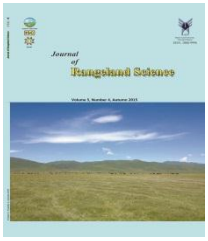

Research and Full Length Article:

Investigation of Chilling Effects on Characteristics of Seed Germination, Vigor and Seedling Growth of *Nepeta* spp. Species

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Abstract. To study the effects of chilling on dormancy breaking and the increased germination characteristics of *Nepeta* (germination percent and speed, seedling length, vigor index, wet and dry weight), a factorial experiment in a completely randomized design with 4 replications was implemented in a laboratory in 2014. Factor A was the chilling treatment in three levels (control, one-month and two-month pre-chilling of wet seeds at 4°C) and factor B was related to the seeds of 10 different species of genus *Nepeta*. After chilling, seeds of control were placed in a germinator for 15 days at 20°C and light-to-dark cycle of 16 hours light (1000 lux) to 8 hours dark. The results showed that chilling treatments had significant effects ($p<0.01$) on germination percent, germination speed, vigor index and fresh weight, but they had no effects on the seedling length. The chilling effects on germination percent showed that one-month chilling had a positive effect on *N. haussknechtii* with 40% and *N. menthoides* with 36.5% and two-month chilling had a positive effect on *N. haussknechtii* with 43%. The two-month chilling effect on germination speed was positive for most species. Generally, one-month chilling had positive effects on the increased seed vigor index of *N. betonicifolia*, *N. haussknechtii* and *N. menthoides*and by 20.48%, 25.33% and 17.99%, respectively as compared to that of control treatment and two-month chilling had positive effects on the increased fresh weight of *N. cataria*, *N. haussknechtii* , *N. pungens*, *N. menthoides* and *N. crassifolia*.

Key words: Chilling, *Nepeta*, Dormancy breaking, Germination

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Introduction

Nepeta is one of important genera of family Labiatae which includes different annual and perennial species. About 250 species of this genus which mainly grow in Asia, Europe and North Africa have been found around the world (Evans, 1996). Over 67 annual and perennial species of genus *Nepeta* grow in Iran and more than 60% of species (39 species) is exclusive to Iran (Mozaffarian, 2006). Several species of this genus are often woody at the base with chamaephytes[1], hemicryptophytes[2] and therophytes[3] growth forms. The leaves are simple with crenate rounded teeth margin. Flowers are dense or separated cymes arranged on stems (Rechinger, 1982). Species of *Nepeta* are different with respect to the essence content and the constituent composition. In the essence of some *Nepeta* species, major components of *Nepeta* lactone isomers have been identified (Fakhr Ranjbari, 1997).

Different parts of these plants are widely used in traditional medicine as anti-seizure, mucokinetic, diuretic, anti-asthmatic, antiseptic, anti cough, sudatory, nutrient, antipyretic and menstrual problems (Amin, 1991). Various constituents have been observed in the essence contents but the most important combination of *Nepeta* species regarding the essence contents is Nepetalactones (Ghannadi *et al.,* 2003) which has different antifungal, antibacterial and anti-virus characteristics (Skaltsa *et al.,* 2000; Aydin *et al.,* 1998).

Seed germination of many plant species could be affected by a mechanism commonly named seed dormancy. Dormancy could be considered as a preventive factor in seed germination even in favorable environmental conditions (Koornneff *et al.,* 2002; Bewley, 1997). Generally, there are two kinds of seed dormancy: physical dormancy (due to hard and impermeable seed coat) and physiological or internal dormancy (due to some physiological conditions, mainly internal hormones) which could delay seed germination (Nasiri *et al.,* 2003). The most common method to break physiological dormancy is cold stratification or moist stratification which in some cases, the use of hormones (Nasiri, 2014; Shariati *et al*., 2002) and chemicals can be replaced with some parts or the whole requirements of stratification (Leadem, 1997). Usually, due to the internal hormonal balance, stratification is useful for germination (increased stimulants concentration and decreased preventives concentration) and is able to break the dormancy (Yamauchi *et al*., 2004). Modares Hashemi (1995) in a study about the effects of chilling on seed dormancy breaking of a few ecotypes of *Prangos ferulaceae* at different times found that the highest germination was obtained after 65 days of chilling at 3-5°C. Nasiri (2014) stated that the required time for chilling the seeds of *Thymus* species is one month to stimulate and increase seed germination. Given that the stratification improves the germination conditions (Graber, 1965; Skordilis and Costas, 1995) and decreases the length of time from planting to germination stages, the seed loss would be minimal. Hence, in order to do a better identification of germination conditions, a test to determine the appropriate time for chilling (cold stratification) to break seed physiological dormancy is essential. The purpose of this study was to evaluate the effects of chilling on seed germination and seedling growth characteristics of some species of the genus *Nepeta* in the libratory.

Materials and Methods

In this research, seeds of 10 species of the genus *Nepeta* with 3 chilling treatments and 4 replications were studied in the

[1] Chaemophytes: Shoots in the air above the ground, but they were less than 25 cm

[2] Hemicryptophytes: Shoots and branches that produce shoots in the following year, on the ground level and protected by dry leaves

[3] Therophytes: Plants that spend the winter as seeds

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laboratory of seed nature seed, gene bank Institute of Forests and Rangelands Management Organization. The treatments involved control, one-month and two-month chilling ones (Table 1). For the control treatment, healthy seeds of 10 species of genus *Nepeta* were equally placed in the sterilized Petri dishes (50 seeds per Petri).

For one-month and two-month chilling treatments, 200 seeds of each species (50 seeds per Petri with 4 replicates) were placed for each treatment. First, seeds were sterilized using sodium hypochlorite 1% (commercial bleach containing 5.5% of active chlorine, 20% volume containing a few drops of liquid soap for 15 minutes). Control and treated seeds (one and two-month at 4°C) were placed in a germinator for 15 days at 20°C and light-to-dark cycle of 16 hours light (1000 lux) to 8 hours dark.

At the end of germination test, number of normal seedlings in each replication was counted and expressed as germination percent. Speed of Germination (SG) was calculated based on the following equation (Maguire, 1962) (Equation 1):

$$SG = \frac{X_1}{Y_1} + \frac{X_2+X_1}{Y_2} + \cdots + \frac{X_n-X_{n-1}}{Y_n} \quad \text{(Equation 1)}$$

Where

X_1, X_2 and X_n are number of seeds germinated on first, second and nth days, respectively.

Y_1, Y_2 and Y_n are number of days from sowing to first, second and nth counts, respectively. Root length and shoot length were measured at the end of germination experiment. Vigor index (VI) was calculated by the following equation (Abdul-Baki and Anderson, 1973) (Equation 2):

$$Vi = \frac{\%Gr \times MSH}{100} \quad \text{(Equation 2)}$$

Where

Vi =vigor index

MSH =mean length of seedling (root + shoot)

Gr% =seed germination percent

The fresh weights of seedlings were weighed using an exact scale. Then, they were immediately placed in aluminum foils and transferred to an oven at temperature of 80°C. After 24 hours, they were weighed again to determine the dry weight. Data for each of characteristics were subjected to the analysis of variance and the means comparisons were made using Duncan method. The statistical analysis was accomplished using SAS_9.

Table 1. Information and geographic location of seed collection area of 10 species of the genus *Nepeta*

Species Name	1000 Seeds Weight	Collection Area	Altitude Above Sea Level (m)
N. haussknechtii	0.75	Ardabil- Razi	1472
N. menthoides	0.44	Ardabil - Meshginshahr	2149
N. glomerul	0.68	Kohgiluyeh and Boyer-Ahmad	2350
N. crassifolia	1.25	Semnan -Shahrud	1362
N. pungens	0.40	Ilam - Darreh Shahr	1620
N. persica	0.55	Yazd - Taft	2634
N. cataria	0.50	Kohgiluyeh and Boyer-Ahmad	2600
N. betoricifolia	0.50	Gilan -Astara	1528

Results

The results achieved by the analysis of variance of factorial experiment showed that the chilling effect was significant on all the germination characteristics except seedling length (P<0.01). The difference between *Nepeta* species was significant (P<0.01). The interactions between species × chilling treatment were also significant for all the characteristics (<0.01) (Table 2).

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Table 2. Analysis of variance of germination characteristics and seed vigor for the genus *Nepeta*

Sources of Variation	df	Germination Percentage	Germination Speed	Root Length (mm)	Shoot Length (mm)	Root to Shoot Length Ratio	Seedling Length (mm)	Seed Vigor Index	Seedling Fresh weight (mg)
Species	9	1005.76**	282.33**	417.20**	735.91**	1.45**	1855.75**	437.40**	3777.97**
Chilling	12	514.16**	369.52**	181.54**	61.85**	1.56**	32.18^{ns}	111.79**	819.82**
Species×chilling	18	112.73**	60.99**	93.41**	62.57**	0.17**	261.43**	60.15**	385.38**
Error	90	6.08	0.81	7.56	5.44	0.02	20.28	2.76	105.79
CV%		10.45	16.29	14.16	10.93	13.91	11.01	16.14	20.50

*, ** and ns= significant at 5% and 1% level and not significant, respectively

Means comparison of treatments

The results of means comparison of pre-chilling treatments showed that two-month chilling of moist seed caused the increase in germination percent and germination speed, shoot length and seedling fresh weight as compared to the control treatment. In addition to the increased germination percent, the one-month chilling treatment was also effective in seed vigor index. In contrast, chilling reduced root length and root to shoot length ratio and had no effects on seedling length (Table 3).

Generally, the results showed that the effects of one-month and two-month chilling treatments on the increased germination were estimated as 30 and 34%, respectively so that they were more effective than control. In addition, the two-month chilling effect on speed of germination (168% more than control) was higher than one-month chilling (Table 3). The results showed that although the impact of treatments on seedling length was equal, the effects of one-month and two-month chilling treatments on the increased shoot length were given as 9 and 12%, respectively so that they were more effective than control (Table 3). The results showed that the effects of one-month and two-month chilling treatments on the increased seed vigor index were given as 38 and 28% so that they were more effective than control; therefore, the effect of one-month chilling on vigor index was higher than the two-month chilling (Table 3). The effects of one-month and two-month chilling treatments on seedling fresh weight were computed as 10 and 20%; they were more effective (Table 3).

Table 3. Means comparison between chilling treatments for different germination characteristics

Treatments	Germination Percentage	Germination Speed	Root Length (mm)	Shoot Length (mm)	Root to Shoot Length Ratio	Seedling Length (mm)	Seed Vigor Index	Seedling Fresh Weight (mg)
Control	19.47 b	3.354 c	21.63 a	19.94 b	1.191 a	41.435 a	8.429c	45.68 b
Two-month chilling	25.30 a	4.209 b	19.24 b	21.73 a	0.959 b	41.382 a	11.64a	50.12 b
Increasing to control	(30%)	(25%)		(9%)			(38%)	(10%
Two-month chilling	26.01 a	8.994 a	17.38 c	22.33 a	0.798 c	39.856 a	10.82b	54.73 a
Increasing to control	(34%)	(168%)		(12%)			(28%)	(20%)

According to Duncan multiple test in each column, means with same letters are not significantly different

Means comparison of species

The comparison of germination characteristics between species showed that *Nepeta hausskne* was the first species and *N. menthoides, N. betonicifolia* and *N. glomerulosa* were next in order. In contrast, *N. crassifoli* with the lowest mean was placed in the last order (Table 4).

The comparison of germination percent between species showed that *N. haussknechtii* and *N. menthoides* with 36 and 29.3% respectively were higher than the others. The lowest germination percent was related to *N. crassifolia* with

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the average value of 10.2% (Table 4). In a comparison between species, the obtained results showed that *N. haussknechtii* had a higher germination speed than the others (Table 4). The results of seedling length showed that *N. haussknechtii* and *N. glomerulosa* with the average values of 55.09 and 55.42 mm had higher length means. The lowest length was obtained for *N. cataria* given as 29.24 mm (Table 4). The highest root and shoot length were observed for *N. glomerulosa* and *N. haussknechtii*, respectively. The highest root to shoot length ratio was observed for *N. crassifolia* computed as 1.59 (Table 4). The comparisons between species showed that *N. haussknechtii* with 19.8 and *N. crassifolia* with 3.4 had the highest and lowest vigor index (Table 4). The comparison between species indicated that *N. haussknechtii* (73.67 mg) as well as *N. glomerulosa* (72.01 mg) and *N. persica* (34.09 mg) had the highest and lowest fresh weights, respectively (Table 3).

Table 4. Comparison between different germination traits of other species of the genus *Nepeta*

Species Name	Germination Percentage	Germination Speed	Root Length (mm)	Shoot Length (mm)	Root/Shoot Length Ratio	Seedling Length (mm)	Seed Vigor Index	Seedling Fresh Weight
N. betonicifolia	27.628 b	9.46 c	23.43 bc	29.40 b	0.83 e	54.023 a	14.84 c	67.90 ab
N. cataria	20.577 c	1.79 f	15.28 f	13.39 fg	1.22 c	29.248 d	5.97 d	37.56 d
N. crassifolia	10.25 e	0.95 g	19.41 de	12.51 gh	1.59 a	32.013 d	3.47 f	34.39 d
N. glomerulosa	28.508 b	2.91 e	27.58 a	28.10 bc	1.06 d	55.098 a	16.31 b	72.01 a
N. haussknechtii	36.25 a	13.94 a	23.63 b	32.01 a	0.74 ef	55.424 a	19.80 a	73.67 a
N. menthoides	29.338 b	8.45 d	17.80 e	27.24 c	0.65 fg	45.039 b	13.59 c	61.51 b
N. persica	13.69 d	1.21 fg	21.19 cd	14.86 f	1.44 b	36.408 c	5.16 de	34.09 d
N. pungens	12.867 d	1.82 f	22.27 bc	20.30 e	1.04 d	42.526 b	5.77 d	42.56 d

According to Duncan multiple test in each column, means with same letters are not significantly different

Effects of species × chilling interactions

The results of factorial analysis of variance showed that the interactions between species and chilling treatments were significant for all the characteristics ($P<0.01$) (Table 2). If the interactions were significant in factorial experiments, the comparison of interactions would be preferred as compared to main effects; therefore, a proper chilling treatment could be recommended for any *Nepeta* species.

The results of means comparison concerning the interactions between species and chilling treatments for different germination characteristics are shown in Figs. 1 to 8. The results showed that the two-month pre-chilling was effective in the germination of all species except *N. pungens* and *N. persica*. Two last species did not require the chilling treatment to increase the germination. The one-month chilling was more effective on *N. betonicifolia* and *N. menthoides* than the other treatments (Fig. 1). The comparison of interactions between species and chilling with respect to the germination speed showed that two-month pre-chilling was significantly effective on *N. betonicifolia, N. glomerulosa* and *N. menthoides* (Fig. 2). Thus, two-month moist chilling of seeds at 4°C is recommended for the cultivation of mentioned species.

The results showed that one-month pre-chilling was effective in the increased root length for *N. menthonic*. In contrast, two-month chilling reduced the root length of all species (Fig. 3). The comparison of interactions between species and chilling treatments for shoot length showed that two-month pre-chilling increased the shoot length of *N. glomerulosa, N. cataria, N. menthoides* and *N. pungen* and the one-month chilling was effective on *N. haussknechtii* and *N. betonicifolia*. The chilling treatments had no significant effects on the increase in shoot length (Fig. 4).

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The comparison results of seedling length showed that two-month chilling and one and two-month chilling treatments were effective in increasing the length of *N. glomerulosa* and *N. betonicifolia*, respectively (Fig. 5). For other species, chilling treatments were not effective on seedling length (Fig. 5). Among germination parameters, the root to shoot length ratio had a reversed trend in comparison with the others. The results showed that this ratio was decreased in all chilling treatment (Fig. 6); it could be due to the effects of chilling treatment on the increased shoot length.

Seed vigor index is one of important indicators in evaluating the germination and plant establishment in the field. The results indicated that two-month chilling was effective in increasing the seed vigor index of *N. glomerulosa* and one-month chilling was effective on *N. betonicifolia*, *N. persica*, *N. menthoides* and *N. haussknechtii*. In contrast, chilling treatments had no significant effects on the seed vigor of other species (Fig. 7). The comparison of interactions of seedling fresh weight showed that two-month prechilling was effective in seedling fresh weight of *N. betonicifolia*, *N. menthoides* and *N. haussknechtii*. The two-month chilling was more effective in seedling fresh weight of *N. betonicifolia*, *N. crassifoli* and *N. cataria* as compared to one-month chilling and control treatments (Fig. 8).

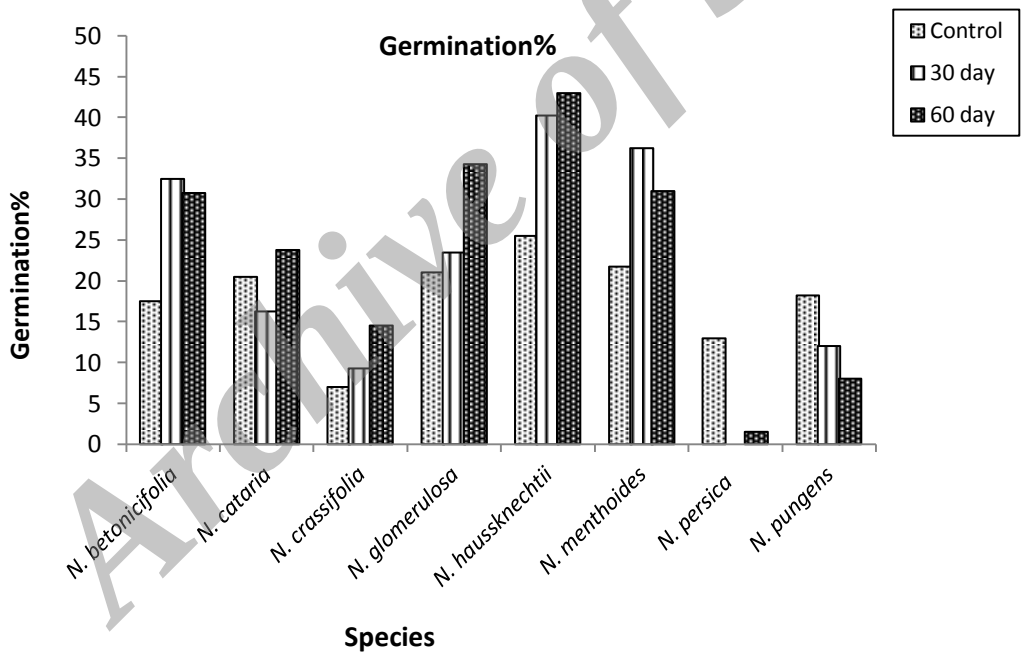

Fig. 1. Mean germination percent of *Nepeta* seed after chilling treatment

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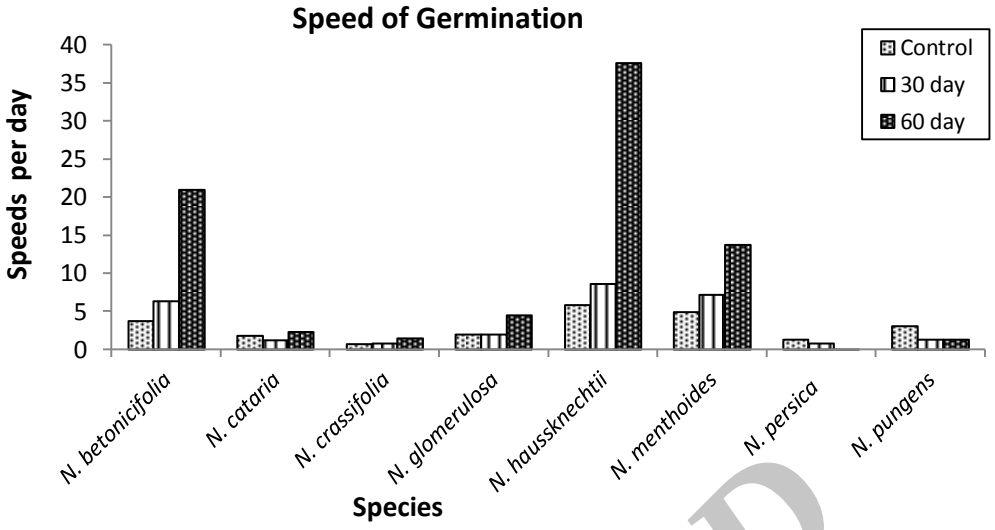

Fig. 2. Mean germination speed of *Nepeta* seeds after chilling treatment

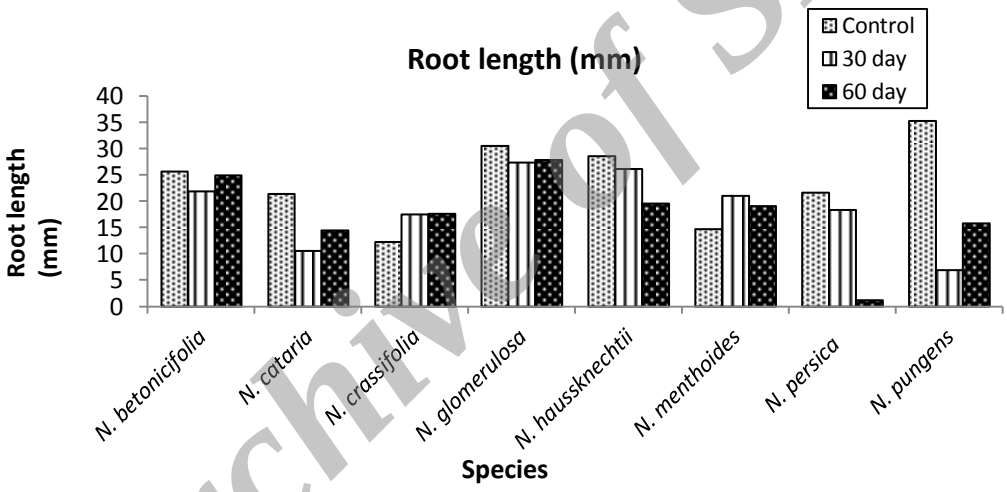

Fig. 3. Mean root length (mm) of *Nepeta* seedlings after chilling treatment

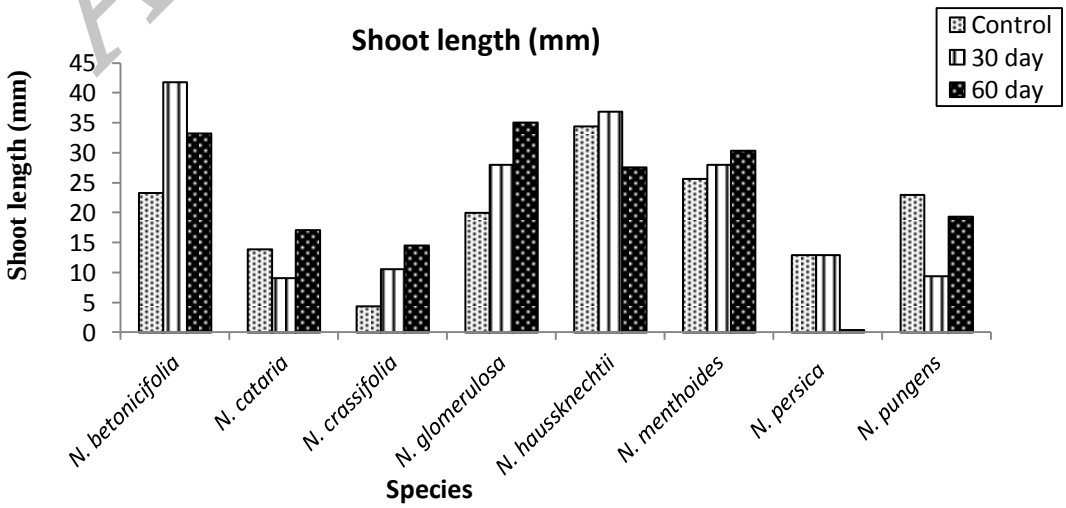

Fig. 4. Mean shoot length (mm) of *Nepeta* seedlings after chilling treatment

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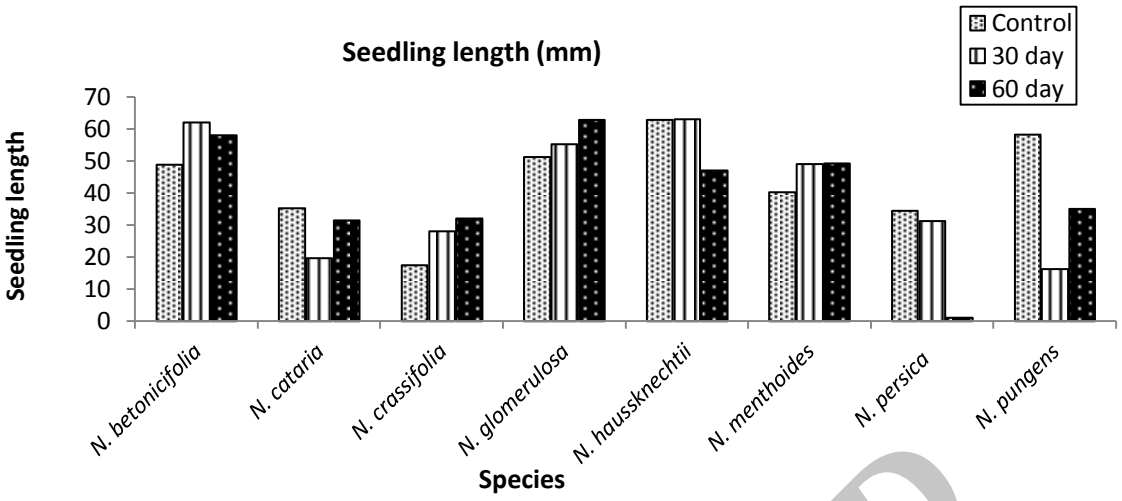

Fig. 5. Mean seedling length (mm) of *Nepeta* seedlings after chilling treatment

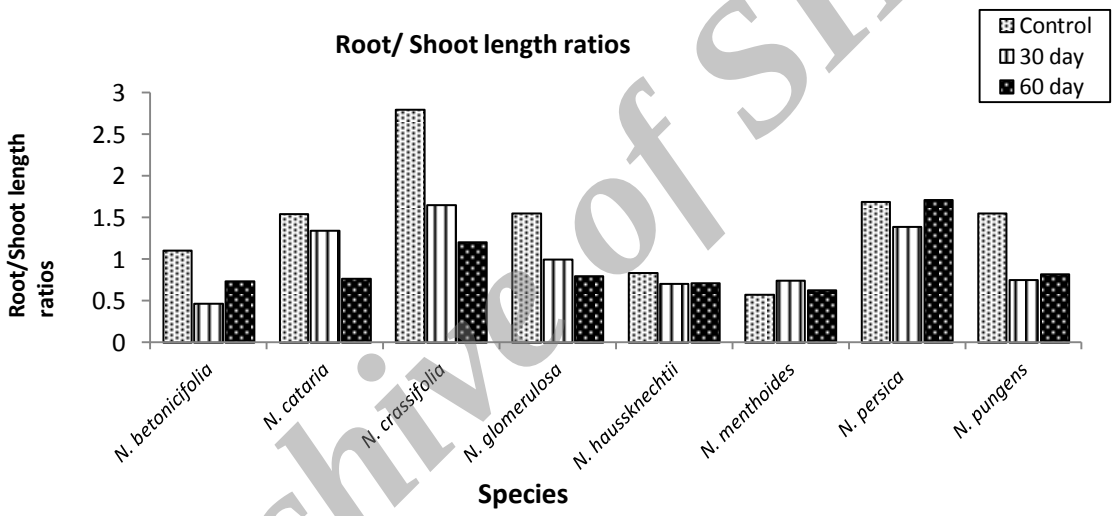

Fig. 6. Root to shoot length ratio of *Nepeta* seedlings after chilling treatment

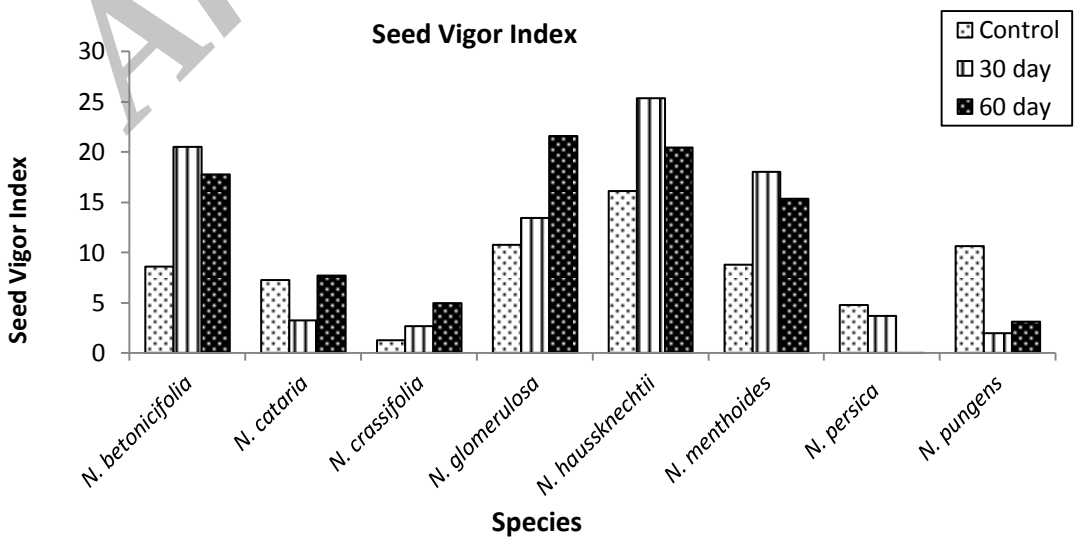

Fig. 7. Mean seed vigor index of *Nepeta* seedlings after chilling treatment

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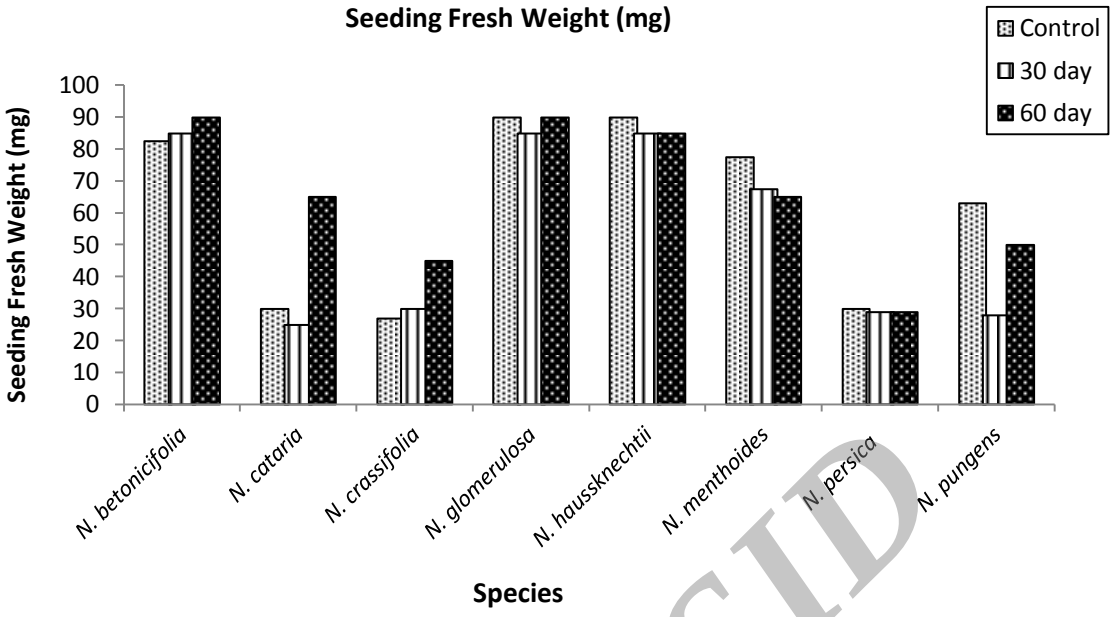

Fig. 8. Mean fresh weight (mg) of *Nepeta* seedlings after chilling treatment

Discussion and Conclusion

The obtained results showed that the germination reactions of *Nepeta* spp. to one and two-month chilling treatments were different. The one and two-month chilling treatments had same effects on *N. betonicifolia* and *N. haussknechtii*. The one-month chilling had a greater impact on germination of *N. menthonic*. The two-month chilling treatment was more effective on *N. cataria, N. glomerulosa* and *N. crassifolia* (Fig. 1). The results reported by Hosienpour Gazviniy *et al.* (2012) showed that chilling treatments had a positive effect on seed germination of *Sature jasahenidica* which is in agreement with our results.

Pederson *et al.* (1993) and Perry (1978) highlighted the importance of germination speed as one of important aspects of seed vigor and considered it as one of the limiting factors in the plants establishment. The germination speed results showed that the species responded to two-month chilling more than the control (Fig. 2); this result was in accordance with those reported by Alizadeh and Jafari (2010). The results of their research indicated that mean germination percent and speed of some *Dactylis glomerata* ecotypes in chilling treatments were higher than the control.

Baskin and Baskin (1999) suggest that a variety of Umbelliferus seeds as well as seeds of other plant species have shown different degrees of physiological dormancy pattern in a manner that chilling can greatly help to break this dormancy of genus *Sambacus (*Hidayati *et al.*, 2000), genus *Dioscora* (Trui and Okagami, 1993) and genus *Cuphea* (Widrelechner and Kovach, 2000).

The species of *N. betonicifolia* and *N. crassifolia* showed a positive effect on shoot length to the chilling treatment. The two-month chilling had a significant effect on shoot length of *N. glomerulosa, N. pungens*, and *N. cataria* (Fig. 4). However, chilling treatments had no significant effects on the root length of species (Fig. 3).

Chilling treatments were effective to eliminate physiological dormancy of some *Nepeta* species. The response of three treatments on seedling length had the same results (Fig. 5). The response of

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control treatment on root to shoot length ratio was higher than the others (Fig. 6).

The one-month chilling treatment had the greatest impact on vigor index of *N. betonicifolia, N. haussknechtii,* and *N. menthoides* (Fig. 7). This result was in accordance with those presented by Nasiri (2014). His results indicated that one-month chilling treatment increased the germination percent of genus *thymus.*

The results showed that two-month chilling treatment had the greatest impact on seedling fresh weight of *N. haussknechtii, N. menthoides, N. cataria,* and *N. crassifolia* (Fig. 8). Regarding this research, some points were concluded:

1) The results of means comparison of pre-chilling treatments showed that two-month chilling of moist seed caused the increased germination percent, germination speed, shoot length and seedling fresh weight more than the control treatment concerning some species of *Nepeta* spp.
2) The one-month chilling effect on vigor index was higher than the two-month chilling.
3) Chilling treatments reduced the root length and root to shoot length ratio and had no effects on seedling length.

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بررسی تأثیر سرما بر خصوصیات جوانه‌زنی بذر، بنیه و رشد گیاهچه برخی از گونه‌های جنس پونه‌سا (*Nepeta* spp.)

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چکیده. به منظور مطالعه اثر سرما بر شکستن خواب، خصوصیات جوانه‌زنی (درصد و سرعت جوانه‌زنی، میانگین طول گیاهچه، شاخص بنیه، وزن خشک و تر) جنس پونه سا، آزمایش فاکتوریل در قالب طرح کاملا تصادفی با ۴ تکرار در شرایط آزمایشگاه در سال ۱۳۹۳، به اجرا در آمد. فاکتور A تیمار سرما در سه سطح (شاهد، پیش سرمای یک ماهه و دو ماهه بذور مرطوب در دمای ۴°C) و فاکتور B بذر ۱۰ گونه مختلف از جنس پونه‌سا بودند. بذرها پس از سرمادهی همراه با شاهد به مدت ۱۵ روز در ژرمیناتور با دمای ۴°C±۲۰ و ۱۶ ساعت روشنایی و ۸ ساعت تاریکی در شرایط استاندارد جوانه زنی قرار گرفتند. نتایج نشان داد که تیمارهای سرمادهی بر درصدجوانه‌زنی، سرعت جوانه‌زنی، شاخص بنیه و وزن تر گیاهچه اثر معنی‌داری ($p<0.01$) داشت، اما بر طول گیاهچه موثر واقع نشد. اثر سرما بر درصد جوانه‌زنی نشان داد که تیمار سرمادهی یکماهه بر گونه ***N. menthoides*** به میزان ۳۶/۵٪ و ***N. haussknechtii*** به میزان ۴۰٪ و سرمادهی دوماهه بر گونه ***N. haussknechtii*** به میزان ۴۳٪ تاثیر مثبت داشته است. اثر سرمادهی دوماهه بر سرعت جوانه‌زنی اکثر گونه‌ها مثبت بود. در مجموع تیمار سرمادهی یکماهه در افزایش شاخص بنیه بذر گونه‌های ***N. betonicifolia, N. haussknechtii, N. menthoides*** به ترتیب به میزان ۱۷/۹۹٪، ۲۵/۳۳٪ و ۲۰/۴۸٪ و تیمار سرمادهی دوماهه بر افزایش وزن‌تر گونه‌های ***N. cataria, N. haussknechtii, N. pungens, N. menthonic, N. crassifoli*** اثر مثبت بیشتری داشت.

کلمات کلیدی: سرمادهی، ***Nepeta***، خواب شکنی، جوانه‌زنی

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