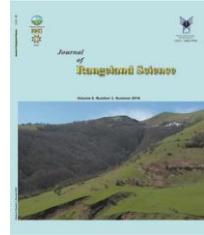


Contents available at ISC and SID

Journal homepage: www.rangeland.ir



Research and Full Length Article:

Effects of Seed Source and Soil Texture on Germination and Survival of *Nepeta asterotricha* as a Medicinal Plant

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Received on: 21/11/ 2015

Accepted on: 15/04/2016

Abstract. *Nepeta* species are widely used as medicine plants because of their anti-spasmodic, diuretic, anti-septic, anti asthmatic anti spasmodic, and tonic effects. *Nepeta* species also have anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activities. Germination and seedling growth are two important stages of the plant development and plant survival. Soil texture is one of the environmental factors, which affects the germination mechanism. In order to evaluate the effects of seed source and soil texture on the germination of *N. asterotricha* as a medicinal plant, an experiment was conducted in a factorial arrangement based on a completely randomized block design with four replications in 2014. The studied factors included different sources of seed collection (Sanij, Dehbala, Dareshir, Tezerjan) and three types of soil texture (Sand, Sandy loam and Silty clay). Results indicated that *N. asterotricha* germination was affected by the origin of seed source (altitude, direction, and slope). Seeds collected from Sanij and Tezerjan had the highest and lowest germination percent, respectively. Among different soil textures, soils with sand and sandy loam texture had the best performance. Results showed significant effects of seed sources and soil texture interaction affects the seed germination percent and germination rate ($p \leq 0.01$). Seed sources had different responses to soil textures indicating that seed collected from different areas may be compatible with specific soil texture. In general, the light soil textures had better effects on seed germination traits of *N. asterotricha*.

Key words: *N. asterotricha*, Seed source, Soil texture, Survival

Introduction

The genus *Nepeta* (Lamiaceae) comprises 280 species that are distributed over a large part of central and southern Europe, West, central, and Southern Asia. About half of the existing species have been recorded in Iran. Topographic factors and diverse edaphic conditions in Iran are responsible for the diversity of microclimate that favors more than 8000 plant species (Haghighi and Mozafarian, 2011).

There are 75 species from *Nepeta*, spp. genus. *Nepeta* species are widely used in folk medicine because of the antispasmodic, diuretic, antiseptic, antitussive, antiasthmatic, ethnobotanical, diaphoretic, vulnerary, antispasmodic, and tonic effects (Micelia *et al.*, 2005). Surveying the essential oil of 38 *Nepeta* species (including native and endemic species) has been reported in Iran (Asgarpanah *et al.*, 2013).

Nepeta species has the anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activities (Dinesh *et al.*, 2010) and can be used as an analgesic, antitussive, carminative, digestive, laxative and sedative one (Formisano and Senatore, 2011). Pardure (2004) investigated chorological and ecological aspects of *N. nuda*. He stated that this species grows in the fields, meadows and glades.

Nadjafi *et al.* (2009) investigated *N. binaludensis* autecology. The results indicated that this plant grows in north-facing slopes with more than 50% and 2300-2700 m elevations. They also found that it prefers the mean annual rainfall ranging from 350 to 370 mm and annual mean temperature of 6 to 7°C and grows in light soils with a neutral pH and low mineral content. However, this plant still undergoes the domestication and one limitation in the large-scale cultivation is the scarcity of scientific research on the germination of *Nepeta* species. Significant factors for seed germination and good seedling establishment in the field are

source of seed and soil type. Devagiri *et al.* (2007) in the assessment of 20 seed sources of *Nepeta* revealed that the seed source had a significant impact on the seedling length, number of shoots on each seedling and seedling biomass. Asgari *et al.* (2015) investigated the chilling effects on characteristics of seed germination, vigor and seedling growth of *Nepeta* spp. species. Their results showed that one-month chilling had positive effects on seed vigor index and two-month chilling had positive effects on seedling fresh weight of *Nepeta* spp. as compared to that of control treatment.

Mamo *et al.* (2006) evaluated the variations in seed and germination characteristics among nine *Juniperus procera* populations in Ethiopia. In the laboratory, the effect of light conditions on seed germination has been tested by incubating the seeds under continuous light or darkness. The results showed that there was a significant difference between the germination rates of nine *Juniperus procera* populations under different light conditions.

Ginwal *et al.* (2005) in the evaluation of *Jatropha curcas* reported that the seed source has a significant impact on the germination characteristics (germination percent, germination rate and germination ability) and seedling growth characteristics such as seedling height and dry weight.

Singh *et al.* (2004) had collected seeds of *Celtis australis* from 13 different sources ranging from 550 to 1980 m above sea level in Central Himalaya, India and concluded that there was a significant difference between different sources in the case of germination rate, average time of germination and germination index.

Cecil and Fare (2002) studied the growth and quality of *Quercus phellos* and *Q. shumardii* in Mississippi and concluded that seedlings originating from southern regions (warmer regions) survived and grew more than seedlings from northern regions (colder regions).

Studying the effects of seed age and soil texture on the germination of some *Ludwigia* species (Onagraceae) in Nigeria showed that some soil types could significantly reduce the germination (Oziegbe *et al.*, 2010). Ebrahimi (2012) reported that germination, growth and uptake of heavy metals by *Hordeum bulbosum* L. in the contaminated soils were affected by dose, dependent responses to the contaminated soils such as reduction in germination rate, height of root and shoot and biomass. Studying the effect of soil texture on *Jatropha curcas* seed germination and survival indicated that the highest rate of germination, germination index and seedling establishment had been found in sandy and sandy loam soils (Ofelia Andrea *et al.*, 2013).

Mohajeri *et al.* (2014) evaluated the seed germination of *Lilium ledebourii* after cryopreservation. They concluded that the cryopreservation technique was an important approach for the long-term preservation of seeds of this endangered species.

Gulshan and Dasti (2012) examined the role of soil texture and depth in the germination of six types of weed seed species of legumes, two grass species, *Fumaria* species and the other one from Rubiaceae species. They stated that the maximum germination rate has occurred in the depth of 2 cm of sandy loam soil. Their results showed a significant positive correlation between the germination and soil texture and soil texture had a key role in the maximum germination rate. Darier *et al.* (2014) investigated the effect of soil type on the allelotoxic activity of *Medicago sativa* L. and showed that the soil type was an important factor in the allelopathic effects of alfalfa on broad beans. Pahla *et al.* (2014) evaluated the effects of soil particle (clay, sand) and various treatments before planting on the germination and vigor of *Acacia sieberana* plant and concluded that the seeds treated with sulfuric acid in 60

minutes and planted in sandy soil had the highest germination percent and the shortest time (6 days) of germination.

According to the investigations, the role of soil texture and seed source in the germination of *N. asterotricha* seeds has not been investigated in Yazd province, Iran. The aim of this study was to evaluate the effects of soil texture and seed source on the germination of *N. asterotricha* species.

Materials and Methods

Study area

N. asterotricha is an endemic plant, which grows only in Yazd province of Iran. Therefore, seeds have been collected from four habitats in the eastern longitudes of 54° 1' 29" to 54° 9' 39" and northern latitudes of 31° 34' 31" to 31° 38' 20". The mean annual precipitation of study area in the past thirty years was 61.7 mm. The mean annual temperature was 18.1°C and maximum and minimum temperatures were +46.5°C and -15.5°C, respectively. This area has a cold and dry climate according to Emberger climate classification and according to the Demarton classification, its climate is ultra-dry cold (Dashtakian and Abolghasemi, 2003). In terms of natural vegetation, the study area is considered as an area without vegetation although some spaced and single plants of *Salsola tomentosa*, *Anabasis setifera*, *Artemisia sieberi*, *Stipagrostis plumosa* and *Launaea acanthodes* are observed on the path of channels (Baghestani Maybodi *et al.*, 2015). The habitats slope of this species ranges from 5 to 80% in the northern, northeastern, western, and eastern slopes (Table 1).

The study of phenology stages of *N. asterotricha* in Dehbala with the elevation about 2779 m above sea level showed that the plant growth begins in late March and continue until late May. Flowering begins in early June but it has been completed gradually during July. Seed formation occurs in late June and

seed maturity date is mid-July. Due to weather conditions, dormancy period

begins in November and will continue until mid March.

Table 1. Topography characteristics and location of *N. asterotricha* (Seed collection)

Source	Altitude (m)	North latitudes	East longitudes	Slope (%)	Geographical directions
Sanij	2503	31° 38' 20"N	54° 01'29"E	10-30	Northeast
Deh bala	2779	31° 34' 58"N	54° 05'25"E	10-30	Northwest
Dareh shir	2485	31° 37' 27"N	54° 01'52"E	5-30	Northeast
Tezerjan	2475	31° 34' 31"N	54° 09'39"E	10-80	East- northeast

Soil selection and Characterization

Three soils were selected with different textures: sand, sandy loam and silt clay. Soil organic carbon (OC) was determined by Walkey-Black method (1934). Soil pH

and electrical conductivity (EC) were determined in the saturated paste. Soil CaCO₃ was measured by titration method with acid, AFNOR (1987) (Table 2).

Table 2. Some physical and chemical properties of soils

Texture	Sand%	Silt%	Clay%	CaCO ₃ %	OC %	pH	EC dSm ⁻¹
Sand	82	10	8	12	0.26	7.40	0.90
Sandy loam	65	23	12	24.90	0.45	7.51	1.10
Silty Clay	20	39	41	37.50	0.81	7.76	1.80

Experimental site and seed sowing

The survey was conducted in the greenhouse of Natural Resources College in Yazd University, Iran in 2014. The experiment was performed in the pots in a factorial experiment based on the completely randomized design with four replications. The first factor was the seed source collected from four habitats (Sanij, Dehbala, Darehshir, Tezerjan) and the second factor was soil texture (sand, sandy loam, silt clay). The soil in each pot was watered daily up to the field capacity of each soil type in order to maintain the constant soil moisture levels in each container.

Data collection

The germinated seeds were accounted daily from the fourth day until 18th day when no further germination was observed. The survival rate was calculated from the number of seedlings surviving for a period of 14 days by the following formula (Equations 1&2&3).

$$GP\% = \frac{\sum G}{n} \times 100 \tag{1}$$

Throneberry and Smith (1955)

Where:

GP%=Germination Percentage

G=the number of germinated seeds

n=the total number of seeds

$$GR = \sum \frac{Si}{Di} \tag{2}$$

Agrwal (2003)

Where:

GR= Germination rate

Si= number of germinated seeds in each count

Di= number of days to the nth counting

$$VI = \%GP \times MSH / 100 \tag{3}$$

Vashisth and Nagarajan (2010)

Where:

VI= seed vigor index

%GP = germination percent

MSH= Mean seedling length (root+shoot)

Allometric coefficient might be used as the drought tolerance. It was estimated using Agrwal (2005) formula (4).

$$AC = \frac{\text{shoot dry weight}}{\text{root dry weight}} \tag{4}$$

Uniformity of germination (GU) is the amount of time it takes to germinate from 10 to 90% of maximum germination so that the less time indicates more uniform germination of seeds. It has been estimated as follows (Soltani and Maddah, 2010):

$$GU = D90 - D10 \tag{5}$$

Where:

GU=Uniformity of germination

D10 and D90, day times that take seed germinate from 10% to 90%.

Statistical analysis

Data analysis was carried out using SPSS16 software and graphs were drawn using excel software. A two-way analysis of variance was performed and means comparisons were made using Duncan method. Normality and homogeneity of variance were tested using Kolmogorov-Smirnov test. Uniformity of germination has been calculated by Germin software.

Results

The results of analysis of variance are presented in Table 3. Results showed that there were significant differences between sources of seeds for germination percent, germination rate, seed vigor index, and allometric coefficient ($p \leq 0.01$). The effects of soil texture were also significant for germination percent, germination rate, seed vigor index, allometric and uniformity of germination ($p \leq 0.01$).

Interaction effects of seed source and soil texture were significant for germination percent and germination rate ($p \leq 0.01$) (Table 3).

Means comparison between different germination traits for three soil textures of *N. asteroides* has been presented in Table 4. The germination percent in the sandy and sandy loam soils was 72.50 and 71.80%, respectively and decreased to 44.40 % in the silt clay soil ($p \leq 0.01$). Germination rate in the sand and sandy loam soils was 12.40 and 12.80, respectively and reached to 5.90 in the silt clay soil ($p \leq 0.01$). The seed vigor indices in the sand, sandy loam and silt clay soils were 31.50, 28.0 and 20.90, respectively. The sand soil texture had been assigned in the first class ($p \leq 0.01$). There was no

significant difference between other traits in different soil textures.

Means comparison between seed origin (Table 5) showed the seeds collected from Tezerjan had the lowest values for germination percent, germination rate, vigor index and uniformity of germination ($p \leq 0.01$). Higher and lower germination percent of 73.3 and 45.8% has been obtained from Sanich and Tezerjan, respectively. Similarly, the germination rate of Sanij seeds was significantly faster than the rate of other regions. The germination rate decreased from 13.5% in Sanij to 9.7% in Dehbala and 9.5% in Dareshir and 8.7% in Tezerjan, respectively ($p \leq 0.01$). In Dehbala, the seeds had more vigor index than the seeds of other regions ($p \leq 0.01$).

The results of Uniformity of germination (GU) showed the significant effect of soil texture ($p \leq 0.05$) (Table 3). Higher values of both D10 and D90 have been obtained in sandy loam soils (Table 4). However, the difference of D90 - D10 was lower for both sandy and sandy loam soils. The obtained values were of the same trends as other seed characteristics ($p \leq 0.05$).

Interaction effects of soil texture and source of seeds were significant for germination percent and germination rate ($p \leq 0.01$) (Table 6) indicating that seed collected from different regions or altitudes had different responses of germination in soil texture. The seeds collected from Sanij and Dehbala had more performance in sandy loam whereas the seeds collected from Dareh shir and Tezerjan had better germination in sand soils (Table 6 and Fig. 1). In contrast for the other traits, the soil texture had the same response for all the regions (Table 6 and Fig. 1). These results showed that the assessment of both soil texture and sources of seed is necessary for the prediction of *N. asteroides* responses.

Table 3. Analysis of variance and the level of MS significant of four seed sources and three soil textures and their interactions of *N. asterotricha* grown in glasshouse

Sources of variation	DF	MS					
		Germination %	Germination Rate	Seed Vigor Index	Allometric Coefficient	D10	D90
Source	3	1507.63**	51.283**	340.07**	1063.56**	1.48 ^{ns}	2.75 ^{ns}
Texture	2	4375.00**	245.84**	576.29**	874.44*	27.58**	41.10**
Source x Texture	6	222.22**	41.79**	97.43	299.57	3.55 ^{ns}	3.47 ^{ns}
Error	36	9.02	1.27	61.6	166.8	1.69	1.94

** Significant at the 1% level, * significant at 5%

Table 4. Comparison between different germination traits for three soil textures of *N. asterotricha* in glasshouse condition

Soil Texture	Germination %	Germination Rate	Seed Vigor Index	Allometric Coefficient	D10	D90
Sand	72.50 ^a	12.35 ^a	31.51 ^a	23.16 ^a	0.88 ^b	4.02 ^c
Sandy Loam	71.87 ^a	12.82 ^a	28.02 ^b	21.10 ^a	3.17 ^a	7.16 ^a
Silty clay	44.37 ^b	5.92 ^b	20.94 ^b	20.73 ^a	0.56 ^c	6.25 ^{ab}

Means with the same letter are not significantly different ($p \leq 0.05$)

Table 5. Comparison between different germination traits of four seed sources of *N. asterotricha* in glasshouse condition

Seed Source	Germination %	Germination Rate	Seed Vigor Index	Allometric Coefficient	D10	D90
Sanij	73.33 ^a	13.51 ^a	25.87 ^{ba}	27.66 ^a	1.96 ^a	5.75 ^a
Deh bala	66.66 ^a	9.73 ^b	32.57 ^a	24.27 ^{ba}	1.73 ^a	6.13 ^a
Dareh shir	65.83 ^a	9.50 ^b	28.39 ^a	20.22 ^{cb}	1.96 ^a	6.00 ^a
Tezerjan	45.83 ^b	8.70 ^b	20.47 ^b	27.66 ^a	1.16 ^b	6.04 ^b

Means with the same letter are not significantly different ($p \leq 0.05$)

Table 6. Comparison between soil textures by seed source interaction effects for different germination traits of *N. asterotricha* in glasshouse condition

Source	Texture	Germination %	Germination Rate	Seed Vigor Index	Algometric Coefficient	D10	D90
Sanij	Sand	82.50 ^b	14.07 ^{ab}	25.94 ^{bcde}	13.87 ^{cd}	0.20 ^c	2.65 ^d
	Sandy loam	95.00 ^a	16.17 ^a	32.09 ^{abc}	9.36 ^d	3.15 ^{ba}	6.26 ^{ab}
	Silty clay	42.50 ^g	10.30 ^c	19.58 ^{cde}	20.27 ^{bc}	1.60 ^{bc}	5.57 ^b
Dehbala	Sand	72.50 ^{bc}	10.21 ^c	40.92 ^a	26.73 ^{ab}	0.40 ^c	4.21 ^c
	Sandy loam	82.50 ^b	15.77 ^a	34.92 ^{ab}	32.68 ^a	3.87 ^a	7.47 ^a
	Silty clay	45.00 ^{efg}	3.21 ^e	21.86 ^{cde}	23.58 ^{abc}	1.92 ^{cb}	5.28 ^b
Dareh shir	Sand	80.00 ^b	10.96 ^c	29.30 ^{abc}	27.75 ^{ab}	1.47 ^{cb}	5.51 ^b
	Sandy loam	72.50 ^{cb}	12.35 ^{bc}	28.44 ^{bce}	20.50 ^{bc}	1.80 ^{cb}	7.58 ^a
	Silty clay	52.50 ^{ef}	5.20 ^{de}	27.44 ^{bce}	24.58 ^{abc}	0.58 ^c	7.06 ^a
Tezerjan	Sand	55.00 ^{de}	14.15 ^{ab}	29.88 ^{abc}	24.31 ^{abc}	1.44 ^{cb}	3.62 ^{cd}
	Sandy Loam	45.00 ^{efg}	6.98 ^d	16.62 ^{de}	21.87 ^{abc}	3.86 ^a	7.32 ^a
	Silty clay	37.50 ^g	4.97 ^{de}	14.90 ^e	14.50 ^{cd}	1.96 ^{cb}	5.75 ^a

Means with the same letter are not significantly different ($p \leq 0.05$)

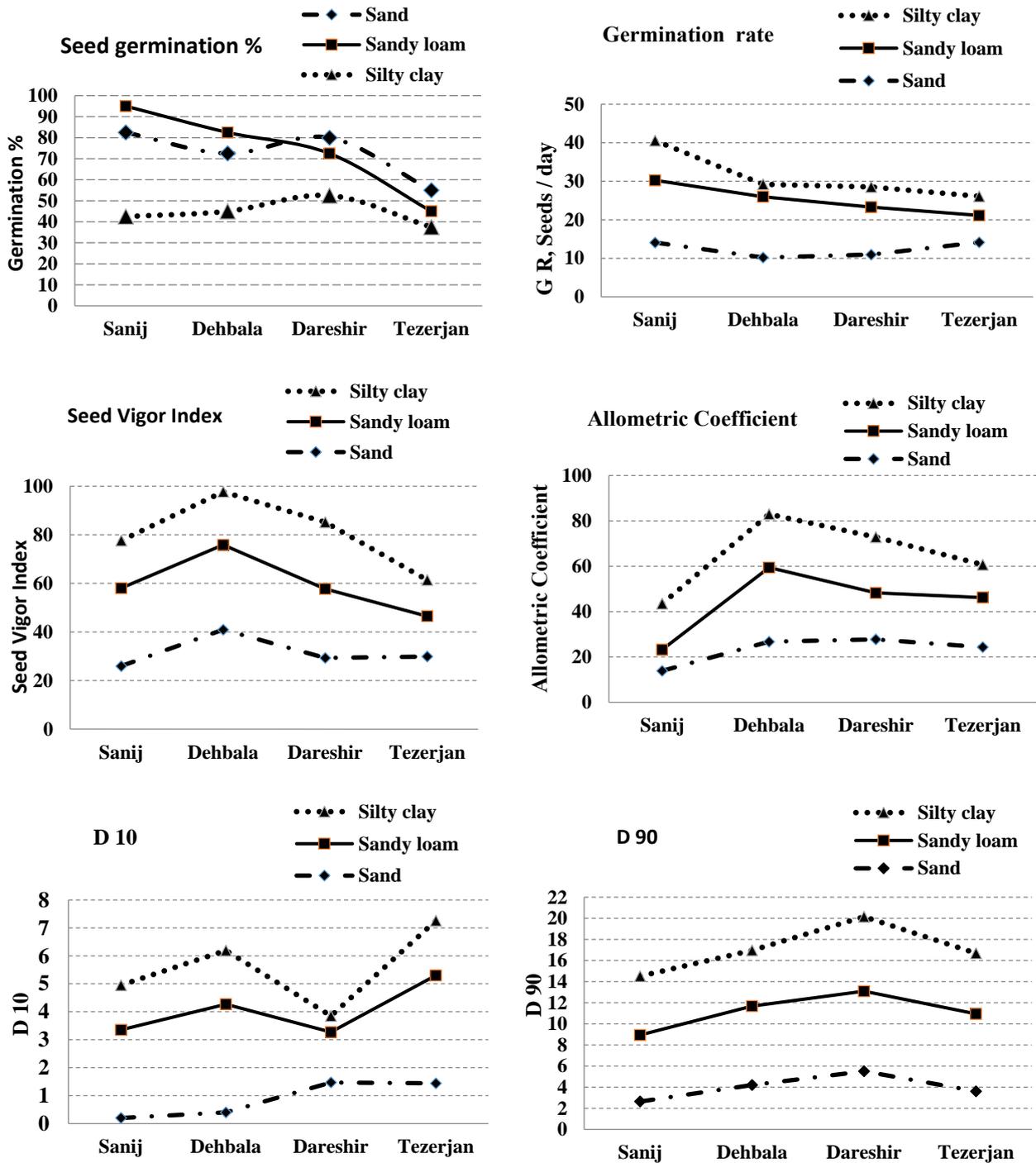


Fig. 1. Trends of soil texture in four seed sources for germination percent, germination rate, seed vigor index, allometric Coefficient, D10 and D90 of *N. asterotricha* in glasshouse condition

Discussion

The obtained results showed that among different soil textures, soils with sand and sandy loam textures had the best performance for *N. asterotricha* germination rate; Ofelia Andrea et al. (2013) found similar results in a study of *Jatropha* germination. Larger pores and

better aeration in sand soil can be two factors of increased germination percent and rate in sandy soils. The results obtained about the seed source indicated that *N. asterotricha* germination was affected by the seed source (altitude, direction and slope) so that Sanij and Tezerjan seeds had the highest and lowest germination percent, respectively. In most

references, it was reported that the seeds of a species collected from different sources or altitudes are different in germination, growth and production efficiency (Isik, 1986; Todaria and Negi, 1995). Ginwal *et al.* (2005) found that the seed source had a significant impact on the germination characteristics. Singh *et al.* (2004) had collected seeds of *Celtis australis* from 13 different sources ranging from 550 to 1980 m above sea level in central Himalaya, India and concluded that there was a significant difference between different sources in the case of germination rate, average time of germination and germination index.

In General, germination characteristics such as germination percent, germination rate, seed vigor index, allometric coefficient and uniformity of germination have been considered as the indicators of seedling growth and development. In addition, the germination percent and seedling length were considered as the criteria for evaluating the vigor index in the early stages of plant growth and in many plant species, the correlation between these characteristics and seed vigor has been proved (Hampton and Tekrony, 1995). In their research, it was found that the amount of these characteristics has been influenced by the seed source (elevation above the sea level, direction and slope) so that with the increased slope percent and geographical direction, the amount of these characteristics decreased steadily. In this regard, Cecil and Fare (2002) had studied the growth and quality of *Quercus phellos* and *Q. shumardii* in Mississippi and concluded that seedlings originating from southern regions (warmer regions) would survive and grow more than seedlings from northern regions (colder regions). Isik (1986) in the study of *Pinus brutia*, and Tabari *et al.* (2006) in the study of *Acer velutinum* showed that the seeds in high elevation (colder regions) germinated more slowly had lower germination percent and smaller seedlings as compared

to the seeds from low and middle elevations (warmer regions). Such differences may be seen in the seed source due to different conditions and environmental resources (nutrition, light or water) which are available for native plants during the growing season (Todaria and Negi, 1995). However, outside the greenhouse, the ability of seed germination is also depended on environmental factors such as length of day, temperature and light quality. The temperature is important so that sometimes, even a slight increase in temperature (during development stage or seed ripening) will significantly affect the ability of seed germination.

Conclusion

Nepeta species has anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activities and can be used as an analgesic, anti tussive, carminative, digestive, laxative and sedative one. Therefore, the conditions of growth and reproduction of this species are important. Seed source and soil type have been found to affect the germination of *N. asterotricha*.

In this research, we found that the amounts of these characteristics have been influenced by the seed source (elevation above the sea level, direction and slope) so that with the increased slope percent and geographical direction, the amount of these characteristics decreased steadily. The results obtained about the seed source indicated that *N. asterotricha* germination has been affected by the seed source (altitude, direction and gradient) so that Sanij and Tezerjan seeds had the highest and lowest germination percent, respectively. Moreover, soil texture significantly affects the germination activity so that among different soil textures, soils with sand and sandy loam textures had the best performance.

Acknowledgements

We are grateful to the Research Council of Natural Resources Faculty of Yazd University, Iran for their support.

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تأثیر منبع بذر و بافت خاک بر سبز شدن گیاه دارویی پونه‌سای کرک ستاره‌ای (*Nepeta asterotricha*)

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تاریخ دریافت: ۱۳۹۴/۰۸/۳۰

تاریخ پذیرش: ۱۳۹۵/۰۱/۲۷

چکیده. گونه‌های مختلف جنس گیاه *Nepeta* در طب سنتی در سطح وسیعی به دلیل داشتن خواص مفیدی مانند تقویت کننده سیستم گوارش، ضد اسپاسم، ضد عفونی کننده و ضد سرفه مورد استفاده قرار می‌گیرد، همچنین این گیاه دارویی فعالیت‌های مهم ضد باکتری، ضد قارچ، ضد ویروس و ضد التهابی نیز از خود نشان داده است. جوانه‌زنی و رشد گیاهچه از مهمترین مراحل نمو گیاه بوده و بقای گیاه وابسته به آنهاست. از طرفی بافت خاک یکی از عوامل محیطی موثر بر جوانه‌زنی می‌باشد. به منظور بررسی تاثیر مبدا بذر و بافت خاک بر سبز شدن بذر گیاه دارویی پونه‌سای کرک ستاره‌ای، آزمایشی به صورت طرح فاکتوریل در قالب طرح کاملاً تصادفی با چهار تکرار در سال ۱۳۹۴ اجرا شد. فاکتورهای مورد بررسی شامل منبع بذر، از چهار منطقه (سانج، ده بالا، دره شیر، طزرجان) و سه نوع بافت خاک (لوم شنی، شنی و رسی سیلتی) بوده است. نتایج نشان داد که عامل مبدا بذر که خود متاثر از ارتفاع، جهت و شیب دامنه می‌باشد نقش مهمی در سبز شدگی و بقاء بذر دارد همچنین نتایج نشان داد که بذرهای منطقه سانج و طزرجان به ترتیب بالاترین و پایین‌ترین درصد جوانه‌زنی را دارند. از میان بافت‌های مختلف خاک، بافت لوم شنی و شنی بهترین عملکرد را از خود نشان دادند. اثرات متقابل بین منبع بذر و بافت خاک بر روی درصد جوانه‌زنی و سرعت جوانه‌زنی در سطح یک درصد معنی داری بود. بطور کلی نتایج نشان داد که بذرهای مناطق مختلف دارای سازگاری با بافت‌های خاصی از خاک می‌باشند، در این تحقیق مشخص گردید که بافت‌های سبک خاک بر خصوصیات یکنواختی جوانه‌زنی، درصد جوانه‌زنی و سرعت جوانه‌زنی گیاه پونه‌سای کرک ستاره‌ای بهترین تاثیر را دارند.

کلمات کلیدی: پونه‌سای کرک ستاره‌ای، منبع بذر، بافت خاک، سبز شدگی