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Autecology of *Nepeta asterotricha* medicinal plant in center of Iran (Case study: Yazd Province)

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Key words: *Nepeta asterotricha*, Autecology, medicinal plant, rare plant, Yazd.

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Abstract

The aim of this study was to determine autecological background of *Nepeta asterotricha* that is a rare endemic medicinal plant distributed in Iran. Site characteristics such as topography, climate, soil, and accompanied plant species were determined. Sampling was done in two different areas and the species phenology, root system, and their presence conditions were examined in the rangeland ecosystem. The results indicated that this species grows in all slopes up to 50% with altitudes of 2500 to 2800 m. The average of annual rainfall changes from 230 to 325 mm and the mean annual temperature is12°C. It was observed that this plant generally prefers Sandy loam and Loamy sand texture, neutral pH and non-saline soils. The content of organic matter is poor in its habitats. Comparing soils in the regions with *Nepeta asterotricha* presence and without it showed that its presence didn't have any significant effects on soil chemical elements. Individual plants height varies from 43 to 63 cm with canopy diameter of 48 to 75 cm. The amount of essential oil changes from1.21 to 1.52. There is no relationship between the essential oil percentage and slope and altitude. *Nepeta asterotricha* growth begins in late March and continues until late May. Flowering begins in early June but it will be completed gradually during July. Seed production occurs in late June and mid-July is the seed maturity time. The main roots penetrated more than 1.2 m into the soil.

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Introduction

To manage a plant species properly, a thorough knowledge about its autecology is required. Autecology is a branch of ecology which study individual species, specially related to their environment (Odum, 1971). Autecological studies are necessary to obtain basic information required for the species management. Interests about plant ecology in desert areas have increased recently (Abdel-Ghani *et al*., 2011). Identifying ecological factors such as soil, topography, climate, and disturbance that influence plant species distribution is essential to conserve, manage and improve rangeland ecosystems (Ajeer&Shahmoradi, 2007; Nautiyal *et al*., 2009). Pouzesh *et al*. (2014) examined *Smyrnium cordifolium* autecology in Iran rangelands and determined its habitats. They found that his species grows in the areas with 350-750 mm rainfall. The average temperature was also between -10 to 35 °C. Dogan and Mert (1998) studied autecology of Vitexagnus-castusas a typical element of Mediteranean macchia vegetation. They expressed that This plant generally prefers neutral, loamy and alkaline soils. It also grows in the soils which are rich in nitrogen and phosphorus with low calcium carbonate and potassium. One of the largest genera of Lamiaceae family (Nepeta genus) belongs to Nepetoideae and tribe Mentheae subfamily, which comprises about 300 herbaceous perennial and rare annual species (Formisano *et al*., 2011). One habitat of Nepeta is from North Africa to Europe and Asia. There are 75 species of Nepeta genus and 39 species are endemic in Iran (Jamzad *et al*., 2002). Nepeta species have anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activity (Micelia *et al*., 2005). Several species of Nepeta can be used as antispasmodic, diuretic, febrifuge, diaphoretic and for tooth, kidney and liver disease (Dinesh *et al*., 2010).

Zarezadeh *et al*. (2007) studied 37 species of xerophytes medicinal plants in Yazd province and found that Apiaceae, Laminaceae, and Solanaceae families had the most number of species. Kaya and Aksaka (2007) studied the morphological and Autecological Properties of *Salvia rosifolia* and found that there is a relation between the quantities of phosphorus, nitrogen and potassium and plant abundance and distribution. The characteristics of *Nepeta oxyodonta* habitat have been investigated in central Zagros Mountains (Mirinejad *et al*., 2014). The results showed that *Nepeta oxidant, usually* grows in the mountain regions with the elevation of 2150 to 2800 meters (above sea level) and the slopes of 10% to 75%. This plant can tolerate the EC from 0.37 to 0.85 µ mho/cm. Surveying the essential oil of 38 Nepeta species (including native and endemic species) have been reported in Iran (Asgarpanah *et al*. 2013). Pardure (2004) investigated chorological and ecological aspects of *Nepeta nuda*. He stated that this species grows in fields, meadows and glades. Nadjafi *et al*. (2009) investigated *Nepeta binaludensis* Autecology. The results indicated that this plant grows in north-facing slopes with more than 50 % and2300-2700 m elevations. They also found that it prefers the average annual rainfall ranging from 350 to 370 mm and temperature of 6 to 7°C and grows in light soils with a neutral pH and low mineral content.

The purpose of this study is to investigate some autecological characteristics of *Nepeta asterotricha* to specify its ecological requirements and successful establishment. *Nepeta asterotricha*, is an endemic plant which grows only on Shirkuh Mountains in Yazd province. There is no study about this plant autecology. In this study, ecological and phenological characteristics of *Nepeta asterotricha* evaluate in its natural habitats.

Materials and methods

The study area

The studied areas are located 30 km southwest of Taft, Yazd province between east longitudes of 54° 1' 29" to 54° 9' 39" and north latitudes of 31° 34' 31" to 31° 38' 20". The average altitude is 2600 m above sea level (Fig.1). The climate is cold semi-arid with the average annual precipitation of 230-325 mm and the mean annual temperature of 12.2 °C. January and July are the months with mean minimum and

maximum temperatures of –17.4 and 33.6 °C, respectively.

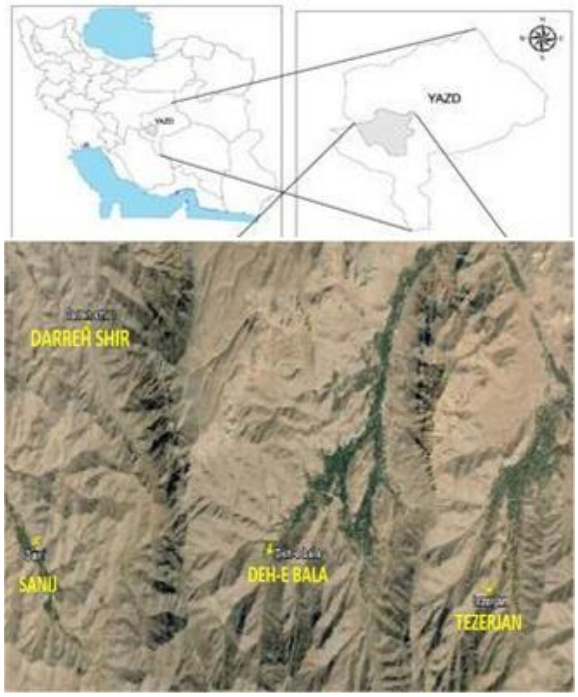


Fig. 1. Location of the study areas.

Methods

Habitats of Nepeta *asterotricha* were found using library resources, publications, consulting experts in medicinal plants and flora and herbarium in Yazd province. Then, field properties such as the coordinates, altitude, direction and percentage of slopes were determined using GPS. Soil study and its chemical composition in each site (4 sites), six profiles were used. Sampling was done in two different areas (under *Nepeta asterotricha* and without it). Soil samples were taken from 0-45 cm depth. In the laboratory, soil chemical properties including organic carbon (OC), total nitrogen (TN), total phosphorus (TP), potassium (K), pH, EC were measured. To determine organic carbon (OC) and nitrogen, Walkey-Black method (1934) and Kjeldahl procedure (Bremner and Mulvaney, 1982) was used, respectively. Soil potassium content was determined by the normal ammonium acetate method. Soil pH and electrical conductivity (EC) were determined (saturated paw method, AFNOR, 1987) by pH meter and conductivity meter, respectively.

To determine phenology and growth duration, from the beginning of growing season, the plant growth area was visited biweekly and vegetative stages of plant growth (including start germination, tillering and Multi-leaf, appearance of flowering stems, fruit development, milky seeds, seed maturity, seed abscission, wilting and drying, temporary regrowth falling dormancy and winter dormancy) were recorded in related forms. Rooting depth was also measured in each soil profile. Finally, seeds germination capacity and seed thousand weights was measured in the lab.

After collecting all data from the sites, analysis was done using SAS software (one-way analysis of variance). Mean comparisons were performed using Duncan's Multiple Test.

To determine the amount of plant essential oil, plant samples were collected in the full bloom stage (flowers, leaves and stems). In order to determine the amount of the essential oil, Samples were dried in the shade and transported to the laboratory. According to international standards, the most common method, steam distillation, was used. To obtain the essential oil, a2-liter distillation flask, distillation column and heater was used. After 2 hours, the essential oils were taken out by asyringe and put into the glass container covered with aluminum foil in the refrigerator and kept at 4 ° C.

Results

Habitat characteristics of the studied species

The results showed that *Nepeta asterotricha* grows at altitudes of 3518-2399 m above sea level. According to 13 years statistics (1392- 1379), the average annual precipitation is 325-230 mm, the average of the maximum and minimum rainfall occur in April (86.4 mm) and September (0.1 mm), respectively. The mean annual temperature is 12.2° C and absolute maximum and minimum temperatures are 33.6° C and -17.4° C in these regions, respectively. Similarly, the warmest month is July and the coldest is January. The habitats slope of this species ranges between 5 to

80 percent and they can be seen in the north, northeast, west and east slopes (Table 1). Overall, the climate is cold semi-arid based on Amberjeh and according to Embrotermic curve. June, July and August are the driest months of the year (Fig. 2).

Table 1. Topography characteristics and location of species growing in sites.

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

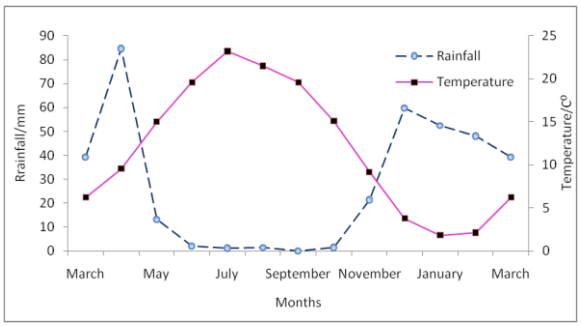
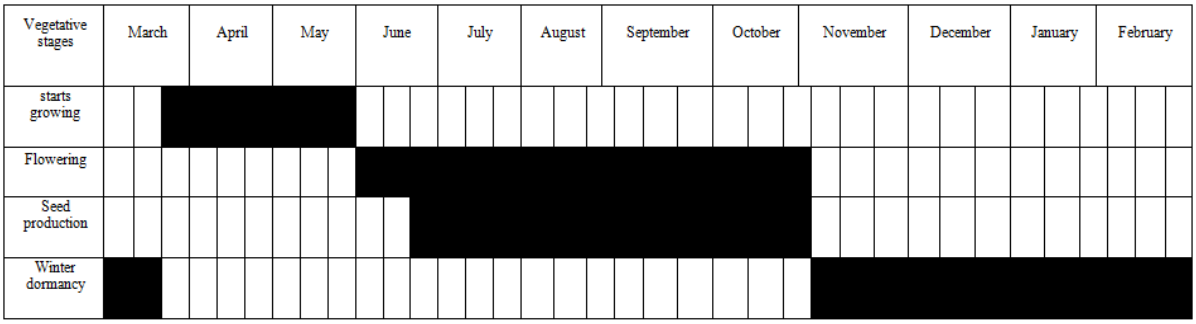


Fig. 2. Embrotermic curve of Dehbala station related to 2000-2013.

Studing phenology stages of *Nepeta asterotricha* in Dehbala with elevation of about 2779 meters above sea level shows that the plant growth begins in late March and continues until late May. Flowering begins in early June but it will be completed gradually during July. Seed formation occurs in late June and mid-July is seed maturity time. Single flowers appear in the bushes before the frost action. Due to weather conditions, plant winter dormancy period begins in November and will continue until mid-March. However, studies in other sites (like Tezerjan, Sanij and Dareshir) which their elevation is less than Dehbala showed that phenological stages (growth, flowering and seed formation, etc.) take place about 15-10 days earlier (table 2).

Table 2. Phenological stages of *Nepeta* asterotricha.



Like any other plants, growth and survival of *Nepeta asterotricha*, during the different stages of growth need different amounts of moisture. This plant root system can penetrate deep into the soil (120 cm) to access water and fight the drought. The root system of the plant in the soil layers has little lateral branches and main root is usually moving forward as a vertical axis into the ground. Lateral roots, are thick and extended horizontal at a depth of 30 to 40 cm of soil and then grow downward. Evaluation of *Nepeta asterotricha* seed viability showed that the plant seeds germinate immediately after they are collected from its habitat. This study also revealed that seeds collected from Sanij have 99% seed viability in comparison to the seeds collected from Tezerjan (93% seed viability), Which are the maximum and minimum seed viability, respectively (Table 3). Grain seeds weight also varies in different areas, so that the grain weight of thousand seeds in Dehbala with 0.6

grams and in Darrehshir with 0.28 grams are maximum and minimum weights, respectively.

Table 3. Some of ecological parameters of *Nepeta asterotricha* in different area.

	Deh-e bala	tezerjan	Sanij	Darrehshir
Height (cm)	63	32	52	46
Seed Thousand weight (g)	0.6	0.3	0.29	0.28
Germination (%)	97	93	99	96
Root length (cm)	110	120	80	120
Spread the roots (cm)	27	35	25	30
Essential oil (g)	1.42	1.5	1.21	1.52

The results showed that the essential oil of *Nepeta asterotricha* in Dehbala, Tezerjan, Sanij and Darrehshiris1.42, 1.5, 1.21 and 1.52, respectively. So, the essential oil of *Nepeta asterotricha* in Darrehshir is the most and in Sanij is the least.

The minimum and maximum electrical conductivity are 0.58 and 1.63 ds/m, respectively, indicating the soils with low salinity. The soil pH values range from 7.5 to 7.99, which are related to alkaline class.

Comparing soil properties in different study areas show that there is a significant difference in the sites related to some chemical properties such as pH and potassium ($p<0.01$). Soil physical properties (sand, silt and clay) have also a significant difference in different study areas (Table 4).

According to the experiments performed in the study areas (Dehbala, Tezerjan, Sanij and Darrehshir), soil acidity ranges between 7.5-7.99 that Dehbala has the minimum (7.5) and Tezerjan has the maximum value (7.99). According to Fig.3, the soil EC values, also have a significant difference in these sites and ranges between0.58in Tezerjanand1.63 in Darrehshir.

Evaluation of soil potassium in these four regions showed that the lowest and the highest amount of potassium are59 ppm for Tezerjan and 167.35 ppm for Dehbala, respectively. Although soil phosphorus levels in these sites vary between 3.56 to 6.48, but there is no significant difference between study areas. Soil organic matter content is low and varies between 0.47 to 0.82. No significant difference was found related to soil organic matter among these areas.

Comparing soils in the regions with *Nepeta asterotricha* presence and without it using Duncan test, showed that the factors like clay, silt, sand, organic matter, phosphorus, potassium, pH and electrical conductivity, don't have a significant difference($p<0.05$). It seems that this species presence has no significant effect on the studied parameters. Soil properties in the two mentioned treatments are given in Table 5.

Table 4. Different soil parameter analysis of variance in studying areas.

	DF	%Clay	%Silt	%Sand	OC %	P (P.P.M)	K (P.P.M)	EC dc/m	PH
Treat	1	43.718 *	4.738 ns	76.095 ns	0.472 ns	0.251 ns	918.211 ns	0.448 ns	0.040 ns
Area	3	134/778**	3392.878**	4416.964 **	0.155 ns	7.569 ns	22437.229**	1.205 ns	0.248 **
Treat* area	3	18.676 *	3.921 ns	11.673 ns	0.109 ns	3.772 ns	866.425 ns	0.025 ns	0.008 ns
Error	10	4.568	29.809	32.449	0.222	4.094	2058.701	0.442	0.014

ns: No significant difference. *: Significant difference at 5 percent. ** Significant difference at 1 percent:

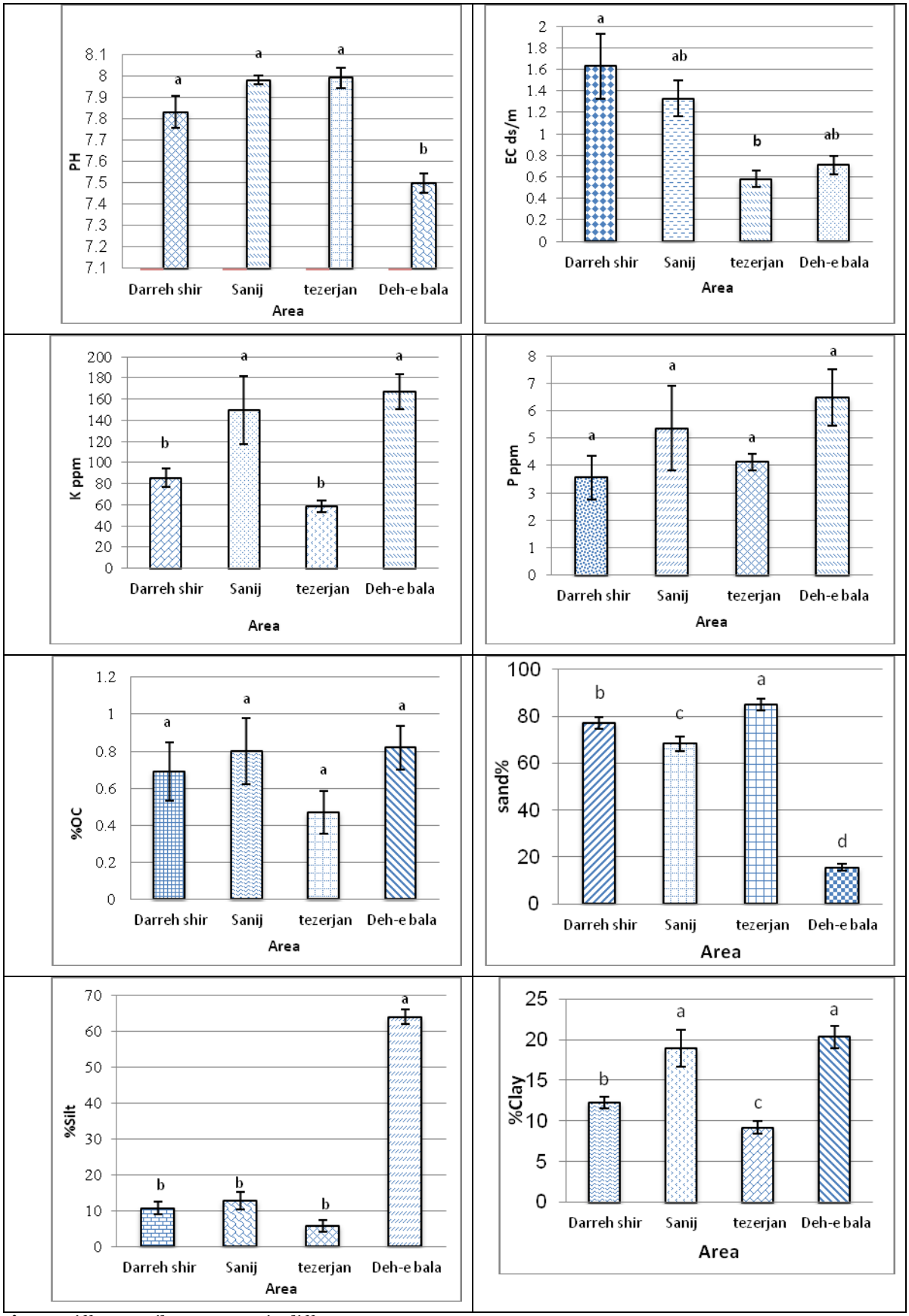


Fig. 3. Different soil parameters in different areas.

Table 5. The effect of N. asterotricha on selected chemical properties of soil.

	Clay %	Silt %	Sand %	OC %	P (P.P.M)	K (P.P.M)	EC (dc/m)	PH
*N. asterotricha*presence	13.58 a	20.20 a	66.19 a	0.91 a	5.20 a	116.12 a	1.3 a	7.8 a
N. asterotricha absence	16.60 a	23.87 a	59.53 a	0.52 a	4.5 a	115.13 a	0.93 a	7.86 a
Error	14.35	23.48	42.93	0.41	15.6	7447.44	0.27	0.01

Vegetation survey showed that *Nepeta asterotricha* is not the dominant species of the region and it grows with several different types of species. Some of these species are listed in Table 6.

Table 6. Some of the Companion species that growing along with *Nepeta asterotricha* in the study areas.

Deh-e bala	tezerjan	Sanij	Darrehshir
Astragalus spp	*Bromus rechingeri*	*Amygdalus lycioides*	*Eryngium bungei*
Acantholimon incomptum	*Cicer spiroceras*	*Atraphaxis spinosa*	*Juncus bufonius*
Acantholimon nigricans	*Echinops aucheri*	*Clematis ispahanica*	*Trifolum alexandrinum*
Acanthophyllum laxiusculum	*Oryzopsis holciformis*	*Cotoneaster persica*	*Echinops aucheri*
Achillea wilhelmsii	*Pimpinella dichotoma*	*Eurotia ceratoides*	*Eurotia ceratoides*
Artemisia aucheri	*Polygonum dumosum*	*Hertia intermedia*	*Rumex dentatus*
Artemisia persica	*Prangos ferulacea*	*Marrubium vulgare*	*Marrubium vulgare*
Berberis integerrima	*Salvia sclarea*	*Salvia macrosiphon*	*Melica jacquemontii*
Carex physodes	*Scutellaria multicaulis*	*Tamarix aphylla*	*Sanguisorba minor*
Cousinia onopordioides	*Stachys inflata*	*Acantholimon incomptum*	*Stachys inflata*
Juncus bufonius	*Stachys obtusicrena*	*Acanthophyllum laxiusculum*	*Stachys obtusicrena*
Marrubium vulgare	*Veronica anagalis*	*Achillea wilhelmsii*	*Trifolum pratense*
Melica jacquemontii	*Cousinia onopordioides*	*Cicer spiroceras*	*Verbascum songaricum*
Melica persica	*Juncus bufonius*	*Cousinia onopordioides*	*Veronica anagalis*
Pimpinella aurea	*Melica jacquemontii*	*Juncus bufonius*	*Batrachiumsphaerospermum*
Silene gynodioica	*Silene gynodioica*	*Polygonum dumosum*	*Nasturtium officinale*
Astragalus albispinus	*Stipa barbata*	*Rosa canina*	*Pennisetum orientale*
Stipa barbata	*Verbascum songaricum*	*Sanguisorba minor*	*Plantago lanceolata*
Trifolum pratense	*Danthonia forsskalii*	*Silene gynodioica*	*Polypogon monspeliensis*
Verbascum songaricum	*Glycyrrhiz aglabra*	*Stachys inflata*	*Nepeta persica*
Ziziphora clinopodioides	*Helichrysum davisianum*	*Stipa barbata*	*Salix aegyptiaca*

Discussion

Study of climatic data (rainfall, temperature and climate type) in Shirkooh catchment shows that *Nepeta asterotricha* grows at the altitudes of 3518-2399 meters above sea level. It seems that high elevation is a limiting factor for this species growth. Nadjafi *et al.* (2009) investigated *Nepeta binaludensis* autecology and stated that this plant grows at an altitude of 2300-2700 m above sea level, While Mazandarani and Lotfi (2013) didn't have expressed any height limits of *Mentha longifolia* growth. Cold semi-arid climate with an average annual precipitation of 230-325 mm and the mean annual temperature of 12.2 ° C can be suitable habitat for *Nepeta asterotricha*. This species found in areas with slope gradient of 5-80% located in the north, the northeast, west and east slope aspects.

According to the results of analyzing soil chemical and physical factors at different growing sites of *N. asterotricha*, it was found that this plant habitat has a soil with moderate to relatively light texture in general. The soils have a relatively high porosity and good permeability, with soil acidity ranges between 7.5-7.99and electrical conductivity ranging 0.58-1.63 ds/m. Nadjafi *et al.*, (2009) also reported a similar

situation about *Nepeta binaludensis*. In different habitats, soil organic matter varies from 0.47 to 0.82, and it is appropriate. The amount of phosphorus in the soil ranges between3.56 and 6.48 ppm and it is placed in poor class. The potassium content of the soil is relatively low, and it is varied between59-167.35 ppm.

The results also showed that the plant has no significant effect on soil chemical properties, such as phosphorus, potassium, organic matter content, pH and salinity, while many studies have shown that plants have many impact on the amount of the chemical elements in soil.

Fattahi *et al.* (2013) stated that areas covered with *Acantholimon olivieri* significantly increased the amount of phosphorus, calcium, nitrogen and carbon in soils and the electrical conductivity and pH is decreased. Fakhireh *et al.* (2010) also showed that nitrogen fixation by *Desmostachya bipinnata* which improve soil physical and chemical properties. The amount of essential oil extracted from 100 g of *Nepeta asterotricha* dry tops in Dehbala (2779 m), Tezerjan (2475 m), Sanij (with elevation of 2503 m) and Darrehshir (2485 m) was 1.42, 1.5, 1.21 and 1.52 grams, respectively. The results showed that plants in Darrehshir with 1.52 and Sanij with 1.21 grams have the most and least amount of essential oil, respectively. In Kashan, three species of Nepeta genus called *Nepeta gloeocephala* Rech. F., *N. sessilifolia* Bunge and *N. laxiflora* have been studied and essential oil amounts of these species was reported (1.3, 0.65 and 0.18, respectively)(Batooli and Safaei-Qummi, 2012). It shows that the amount of *Nepeta asterotricha*'s essential oil is considerably more than its amount in *N. sessilifolia* Bunge and *N. laxiflora*.The results showed that there is no logical relationship between growth elevation and the amount of *Nepeta asterotricha*'s essential oil. Nadjafi *et al.* (2009) investigated *Nepeta binaludensis* autecology and declared that there is no relationship between the essential oil of this species and growing elevation and slope gradient. Also, Kalvandi *et al.* (2013) studied essential oil of *Thymus kotschyanus* Boiss in Iran and stated that essential oils have no significant correlation with growing elevation. But Bakhshi Khaniki *et al.,* (2010) investigated the effect of habitat conditions on quantity and quality of *Ziziphora tenuior* (*Ziziphora clinopodioides* Lam.) essential oil. They have found that habitat conditions cause significant differences on the efficiency of essential oils ($p<0.01$).

In this study, the plant root system includes the main root and secondary or lateral roots. The main roots penetrate to a depth of 30 to 40 cm depending on the soil texture and moisture and then spread horizontally. Root length reaches up to 120 cm. Surface roots of this plant are extended in a form of table-like to a depth of 5 to 10 cm.

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