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Original Article

Investigation of Altitude on Morphological Traits and Essential Oil Composition of *Nepeta pogonosperma* Jamzad and Assadi from Alamut Region

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Abstract

Nepeta is one of the biggest geniuses of Lamiaceae family which *N. pungens, N. binaludensis, N. isphanica, N. pogonosperma* and *N. bracteata* has been used traditionally in Iran. *Nepeta pogonesperma* is one of the endemic *Nepeta* species in Alamut region (Qazvin Province). So in this research the aerial parts of *Nepeta pogonesperma* in full flowering stage were collected from Kheshchal altitudes of Alamut (2400, 2600 and 2800 m). Some main morphological characters of plant were measured. Essential oil were obtained by hydro distillation (Clevenger apparatus) and were analyzed by GC/MS. Results were showed that in high altitude (2800m) the yield and quantity of the essential oil components of plant were increased and the 1,8-cineole had the highest content (80.7%). 19 compounds were identified in the essential oil of this plant, Such as α-Pinene, Sabinene, β-Pinene, Myrcene, δ-3-Carene, ρ-Cymene, Limonene, 1,8-Cineole, Cis-Sabinene hydrate, Terpinolene, Linalool, Cis-p-Menth-2-en-1-ol, Pinocarvone, Borneol, Terpinen-4-ol, α-Terpineol, 4aα-7α-7aβ-Nepetalactone, α-Humulene and Viridiflorol. Variance analysis revealed that there was a significant difference between altitudes in the weight of leaf, stem and flower and total plant weight and oil percentage ($\alpha \leq 0.01$). According to the means comparison of the highest leaf and flower weight were belonged to 2400 and 2600 m with 63.7 and 35.7g, respectively. The maximum amount of oil percentage and 1,8-Cineole were observed in 2400 m with 0.55 and 80.7%, respectively. In this research, there were many changes in altitudes in essential oil combinations, which can be increased the production and performance of Nepetalactone, by applying nature-generated modeling factors.

K**eywords:** Altitudes level, Habitat, 1,8-Cineole, Nepetalactone

Introduction

Plant growth and production of secondary metabolites and their compounds in a specific habitat is affected by environmental factors and climatic condition. Sudden changes of environmental factors especially moisture, temperature and altitude, make changes in the production of secondary metabolites. In other words, sever stress put the plant life in jeopardy and weaken it, stress make the plant to response it and produce secondary metabolite at an optimal level. In this regard, local patterns should be indentified and modeled, and intended medicinal plant must be fostered based on the obtained information [1]. Natural habitat specification with high production capacity can be used as a proper pattern for providing cultivation condition in farm and produce

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essential oil with high quality [2]. According to an investigation on *Thymus kotschyanus* Bioss. harvesting in full flower stage from the southern slopes of Alborz in six altitudes (1800-2800 m), altitudes level significantly affected essential oil percent and main components [3]. Investigation the effect of height on secondary metabolite of *Urtica dioica* in natural habitat of three regions (0, 800 and 1800 m) showed that there was significant difference between percentage of all components other than Linalool[4]. According to Dehghan *et al*. [5] report about *Ziziphora clinopodioides* Lam. subsp. *rigida* (Boiss.), essential oil yield in lower altitude was more than higher altitude. Sought to examine the effect of altitude (2400, 2600 and 2800 m) on oil yield of *Thymus vulgaris* in Damavand region, the lowest height was diagnosed the best habitat for achieving the best quality and quantity of essential oil [6].

Nepeta is one of the biggest geniuses of Lamiaceae family with 400 species [7] which 67 species of it have been found in Iran [8]. *N. pungens*, *N. binaludensis, N. isphanica, N. pogonosperma*and *N. bracteata* are medicinal species which are mostly used in Iran [9]. In traditional medicine, this genus is used as antiseptic, astringent, relief skin itching in children, snake and scorpion stings [10], antispasmodic, anti-asthmatic, anti-paralysis [11], diaphoretic, wound healing, antipyretic and pain reliever [12,13]. Some Iranian species of *Nepeta* is used for neurological, respiratory and gastric disorders [14]. Antibacterial activities of *Nepeta* species (*N. cataria* L., *N. granatensis*Boiss., *N. atlantica* Ball., *N. tuberosa* L.) can be related to the major mono-terpenoids components like Nepetalactone. Due to high Nepetalactone in *Nepeta pogonosperma* [15] and extensive use of it in supplying of raw materials for anti-cancer and anti-oxidant drugs, necessity of research and development of cultivation of species with Nepetalactone for effective steps to be taken in replacing chemical drugs is felt.

Material and Methods

Nepeta pogonosperma Jamzad and Assadi was harvested in full flowering stage from Alamout habitat in summer of 2015, which is located in Kheshchal altitudes of Qazvin Province, Iran. Random plot sampling design was used for harvesting from height of 2800, 2600 and 2400 m altitude with three replications and 50 m intervals in each height class [16]. The size of used plots was 10×10 m^2, and there was 36 – 46 plant in each plot which all of them were studied. Plant height, canopy perimeter, main stem diameter, the highest internodes, inflorescence number, the largest inflorescence and leaf number of each plant were recorded. Length and width of 30 large leaves per plant was measured.

Essential oil Extraction

Flowering shoots were harvested from various height classes, and transferred to the laboratory. Samples were dried at shade and room temperature. ،he essential oil was obtained by hydro distillation method (Clevenger apparatus) after 2h and were analyzed by GC/MS. [17].

Gas Chromatography - Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used . The column was same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V.

The identity of the oil components was established from their GC retention indices, relative to C7- C25 n-alkanes, by comparison of their MS spectra with those reported in the literature [18, 19, 20], and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Retention Indices Calculating and Components Identification

In this experiment, a gas chromatography (Shimadzu model) with DB-5 column and a gas chromatography (Varian model of 3400) connected to a mass spectrum were used. After essential oil injection, the components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature [20].

Obtained data were tested for normality, and then analyzed using SAS software in completely randomized design.

Result and Discussion

Variance analysis indicated (Table 1) that harvesting from various regions (altitude levels) significantly affected canopy perimeter, inflorescence number, stem number, leaf number, leaf length and width (α≤0.01), and main stem number (α≤0.05).

There was significant difference between altitudes of habitat on weight and oil percentage (Table 2), so that the effect of various altitudes were different for leaf weight, stem weight, flower weight, total plant weight and essential oil percentage (α≤0.01).

According to means comparisons (Table 3), the second region (2600 m) had the biggest canopy perimeter, the highest inflorescence, stem and leaf number with 210 cm, 143.3, 195.6, 2456 n/per plant, respectively. the maximum of stem diameter was observed in the first region (2800 m) with 0.59 cm. The highest leaf width and length was belonged to 2600 m altitude with 15.6 and 40.3 mm, respectively.

Means comparison of dry weight and essential oil percentage showed (Table 4) that leaf weight in 2400 m altitude was the highest with 63.7 g; thereafter 2600 m had the maximum amount of leaf weight with 62.8 g. The highest stem weight was observed in 2600 m with 123,8 g. The maximum amount of flower weight was belonged to the second region (2600 m) with 35.7 g. The highest essential oil percentage (0.55%) was observed in the third region (2400 m).

According to the results in Table 6 means comparison between different altitudes indicated that the maximum percentage of 1,8-cineole was observed in 2400 m with 80.7%. The highest amount of α-pinene and sabinene were in 2400 m with 1.13 and 4.67%, respectively. ρ-cymene was the maximum in 2600 m with 3.73%.

The second region (2600 m) had the maximum percentage of β-pinene (1.44%). The highest percentage of cis-sabinene hydrate was found in 2800 m with 1.25%. The second region (2600 m) had the maximum percentage of terpinolene with 2.88%.

Table 1a Variance analysis of altitude habitat effect on morphological traits of *Nepeta pogonosperma*.

S.O.V	df	Mean squares									
		Plant height	Canopy perimeter	Main stem diameter	the highest internodes	Inflorescence number	the largest inflorescence	Stem number	Leaf number	Leaf length	Leaf width
Altitudes level	2	96.4^{ns}	3230.7**	0.01*	0.19^{ns}	8725**	5.3^{ns}	19075**	2412067**	366.7**	36.3**
Error	6	41.8	110.5	0.001	0.14	413	4.5	905.3	58313.6	5.6	1.4
CV (%)	-	11.8	6.1	6.4	11.1	24.8	14.2	28.03	16.3	8.4	9.6

^{ns}, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 2 Variance analysis of altitude habitat effect ondry weight and essential oil percent of *Nepeta pogonosperma*.

S.O.V	df	Mean squares				
		Leaf weight	Stem weight	Flower weight	Total plant weight	Essential oil percentage
Altitudes level	2	2354.5**	9337.8**	546**	37768.3**	0.03**
Error	6	76.5	211.8	51.2	47.04	0.00008
CV (%)	-	18.5	21.09	29.2	12.7	6.7

^{ns}, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 3 The effect of altitude habitat on morphological traits of *Nepeta pogonosperma*

Altitudes level (m)	Plant height (cm)	Canopy perimeter (cm)	Main stem diameter (cm)	The highest internodes (cm)	Inflorescence number (n/per plant)	The largest inflorescence (cm)	Stem number (n/per plant)	Leaf number (n/per plant)	Leaf length (mm)	Leaf width (mm)
2800	49 a	153.6 b	0.59 a	3.2 a	43.3 b	15 a	40.6 b	693 c	18.6 c	8.7 c
2600	54.3 a	210 a	0.56 a	3.6 a	143.3 a	16.3 a	195.6 a	2456 a	40.3 a	15.6 a
2400	60.3 a	152.6 b	0.47 b	3.2 a	58.3 b	13.6 a	85.6 b	1290 b	25.6 b	8.7 b

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 4 The effect of altitude habitat on dry weight and essential oil percent of *Nepeta pogonosperma*.

Altitudes level (m)	Leaf weight (g/per plant)	Stem weight (g/per plant)	Flower weight (g/per plant)	Total plant weight (g/per plant)	Essential oil percentage (%)
2800	14.7 b	12.3 c	8.8 b	46.2 c	0.33 c
2600	62.8 a	123.8 a	35.7 a	263.8 a	0.42 b
2400	63.7 b	70.9 b	24.1 a	46.2 b	0.55 a

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 5a Variance analysis of altitude habitat effect on percent of essential oil components of *Nepeta pogonosperma*.

S.O.V	df	Mean squares								
		α-pinne	sabinene	β-pinene	myrcene	δ-3-carene	ρ-cymene	limonene	1,8-cineole	cis-sabinene hydrate
Altitudes level	2	0.4**	3.3**	1.03**	0.04**	0.08**	5.3**	0.32**	384.5**	0.66**
Error	6	0.004	0.06	0.02	0.009	0.007	0.02	0.001	2.5	0.002
CV (%)	-	9.4	6.8	17.1	9.9	5.8	5.8	7.5	2.3	6.6

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 5b Variance analysis of altitude habitat effect on percent of essential oil components of *Nepeta pogonosperma*.

S.O.V	Df	Mean squares									
		terpinolene	linalool	cis-p-menth-2-en-1-ol	Pinocarvone	Borneol	Terpinen-4-ol	α-Terpineol	4aα-7α-7aβ-Nepetalactone	α-Humulene	Viridiflorol
Altitudes level	2	3.8**	18.4**	0.37**	0.18**	2.08**	1.08**	2.6**	13.5**	0.31**	0.14ns
Error	6	0.01	0.01	0.006	0.01	0.006	0.01	0.01	0.01	0.002	0.07
CV (%)	-	5.9	4.5	13.4	7.7	8.5	10.9	8.1	5.5	11.2	27.6

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

According to the results in Table 5, harvesting region (altitude levels) had significant effect on essential oil component, so that 19 identified component was significantly affected by regions altitude levels (α≤0.01).

Linalool was the highest percentage in the first region (2800 m) with 5.56%. Pinocarvone (1.8%), borneol (1.88%),terpinen-4-ol (1.66%), 4aα-7α-7aβ-Nepetalactone (4.7%) and α-Humulene (0.83 %) had the highest percentage in the height of 2800 m altitude. The maximum percentage of α-terpineol was belonged to 2600 m with 2.3 %.

Variance analysis indicated that altitude had significant effect on morphological trait, dry weight and essential oil percentage. Means comparison revealed that the highest inflorescence number, stem number, leaf number, stem weight, flower weight, leaf width and length was observed in the second region with of 2600 m; therefore, it seems that height of 2600 m is the best condition for growth.

Perhaps existence of water deficit and heat stress in lower attitude or wind and light stress in higher attitude is the main reason of this result. *Thymus kotschyanus* Bioss. harvested from habitat indicated that the highest and lowest percent of essential oil was obtained from the minimum (1800 m) and maximum (2800 m) height, respectively.This contradictoriness can be related to the nature of *thymus* tolerance to drought and other stresses in comparison with *Nepeta*. Results were different with Jamshidi *et al*. research on *Thymus vulgaris* [6]. According to the mean comparison of dry weight and essential oil percentage, the maximum amount of leaf weight (63.7 g) was belonged to 2400 m; thereafter 2600 m had higher amount with 62.8 g. the highest stem weight was observed in 2600 m with 123.8 g.

Table 6 The effect of altitude habitat on essential oil components percentage of *Nepeta pogonosperma*.

	Essential oil Components (%)	Altitude levels (m) 2800	2600	2400
1	1,8-cineole	58.27 c	66.8 b	80.7 a
2	Linalool	5.56 a	1.97 b	0.81 c
3	4aα-7α-7aβ-Nepetalactone	4.7 a	2.1 b	0.6 c
4	Sabinene	3.53 b	2.56 c	4.67 a
5	*ρ*-cymene	3.09 b	3.73 a	1.17 c
6	Terpinolene	2.74 a	2.88 a	0.84 b
7	Borneol	1.88 a	0.45 b	0.43 b
8	Pinocarvone	1.8 a	1.3 b	1,54 b
9	Terpinen-4-ol	1.66 a	0.75 b	0.53 c
10	*α*-Terpineol	1.2 b	2.3 a	0.43 c
11	cis-sabinene hydrate	1.25 a	0.45 b	0.41 b
12	cis-p-menth-2-en-1-ol	0.93 a	0.62 b	0.22 c
13	*β*-pinene	0.82 b	1.44 a	0.27 c
14	*α*-Humulene	0.83 a	0.33 b	0.22 c
15	Viridiflorol	0.65 a	0.37 a	0.21 a
16	*δ*-3-carene	0.52 b	0.58 a	0.26 c
17	*α*-pinne	0.52 b	0.47 b	1.13 a
18	Myrcene	0.45 a	0.21 b	0.24 b
19	Limonene	0.37 b	0.93 a	0.35 b

Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Flower weight in 2600 m showed the maximum amount with 35.7 g; thereafter height of 2400 m had higher flower weight (24.1 g) than 2800 m. The highest essential oil percent (0.55%) was observed in 2400 m.

Significant difference between means of Table 5 revealed that altitude levels treatment should be noted in the plant domestication and pays more attention to this factor. Various altitude significantly difference affected oil components which these differences shows height importance and possibility of increasing the desired component in essential oil by crop improvement factors. Height of 2800 m showed the highest percentage (4.7%) of 4aα-7α-7aβ-nepetalactone. The highest percent among *Nepeta pogonosperma* components was belonged to 1,8-cineole; therefore, attention to this component is more important than the others.

Altitude is one of the important factors to changing of ecosystems so that regarding to increasing or decreasing of it, some environmental elements, including temperature, relative humid, wind speed, available water, and radiation rate will be changed, therefore, changes in growth and essential oil composition of the plant are observed at different altitudes. In this research, there were many changes in altitudes in essential oil combinations. Due to the purpose of the plant essential oil and its specific composition, various factors can be investigated to model the altitude factors, to optimize the performance of that particular compound.

In the case of *Nepeta pogonosperma*, the main goal is Neptalactone, at an altitude of 2800 m, it was more than two other heights examined. Therefore, using effective factors from different heights, it is possible to change the quality and quantity of medical plants in field conditions. Thus, by combining the factors at a height of 2600 m, that increased the growth and yield of the plant by factors that increase the Nepetalactone at 2800 m, can produced plant with high yield and desirable Nepetalactone..

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