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Influence of climatic factors on essential oil content and composition of 20 populations of *Nepeta binaludensis* Jamzad from Iran

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Abstract

Nepeta binaludensis Jamzad is an endemic and rare perennial plant belonging to the Lamiaceae family, which grows in a limited area in Binaloud Mountain in northeast of Iran. In this study to evaluate the diversity of 20 populations (localities) of *N. binaludensis* and the influence of environmental factors on essential oil (EO) content and composition, the plant aerial parts were collected at the full flowering stage. The plant materials dried in shade and subjected to hydro-distillation for obtaining their EOs. Analysis of the EO was carried out using GC and GC/MS. The oil yields were varied from 1.2 to 4.9%. Classification of populations was made based on EO compounds. The results of mean comparison between populations belong to different habitats showed that the populations of Darood and Friezy with average values of 4.91 and 1.2% had the highest and lowest EO yield, respectively. Twenty-two compounds were identified in the oils with 1,8-cineol (25.4–59.0%), 4aα,7α,7aα-nepetalactone (13.8–55.1%), myrcene (2.3–5.5%) and p-cymene (1.1–5.7%) as the main components. Result of correlation analysis showed that the oil yield was positively correlated with precipitation and negatively with temperature. In addition, 1,8-cineole was positively and nepetalactone was negatively correlated with altitude. Cluster analysis by Ward method categorized the populations into two groups. The major compound of the oils in cluster 1 was nepetalactone (with an average of 37.9%), while the oils in cluster 2, contained higher percentage of 1,8-cineole (52–59%). Most of the populations in cluster 2, were originated from high, cold, rainy, and steep areas.

Highlights

By increasing the altitude, changes in growth and essential oil composition of the plant are observed

Result of correlation analysis showed that essential oil yield was positively correlated with precipitation, and negatively with temperature. In the present study the populations with high-amount of nepetalactone and 1,8 cineole were identified,

The populations of cluster 2 with higher amount of 1,8-cineole, were growing in the high altitude, cold, rainy and sloping areas

Keywords *Nepeta binaludensis*, Essential oil, 1,8-cineole, Nepetalactone, Multivariate analysis

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Introduction

The genus *Nepeta* L. comprises approximately 300 species, widely distributed in Eurasia. It is one of the largest genera of subfamily Nepetoideae, family Lamiaceae in southwestern Asia. Iran is one of the main origins of this genus with 79 endemic species (ca. 77%) (Jamzad et al. [11]).

Nepeta binaludensis Jamzad is an endemic and rare perennial aromatic herb which is distributed in a limited area in the Binalud mountains in northeast of Iran [18]. It grows wild in habitats with 2300 to 2700 m altitude, 350–370 mm annual rainfall and annual mean temperature of 6–7 °C [17, 18].

The medicinal properties of *Nepeta* species are usually attributed to their essential oil (EO) and flavonoids. Literature review showed medicinal properties of several *Nepeta* species in Iran such as, *N. ispahanica*, *N. binaloudensis, N. bracteata, N. pogonosperma, and N. pungens*. Some other species like *N. crispa* are used as culinary herbs [11]. There are some studies showing the effects of environmental parameters such as temperature, moisture and soil on EO content and composition of aromatic plants (Karousou [12]).

There is no report about the effect of climatic factors (altitude) on its EO yield and composition of *N. binaluednsis*. However, there are some studies that conducted in specific areas with no variation in the altitude. Rustaiyan and Nadji [19] found 16 components in the EO of *N. binaluednsis* with 1, 8-cineol (42.3%) and nepetalactone (25.2%) as a major component, Najafi et al. [17] identified 18 constituents with 1, 8-cineol (73.2–77.8%) as a major component of *N. binaludensis*. However, the latter authors did not report nepetalactone isomers as the main compounds of the EO. Mohammadpour et al. [16] found 1,8-cineol (68.3%) as the main component of *N. binaluednsis* EO. Asgarpanah et al*.* (2003) reported nepetalactone (25.0%) and 1,8-cineole (42.0%) [4], Gkinis et al. [9] found nepetalactone (25.0%) and more recently, Amirmohammadi et al. [2] reported nepetalactone (59.7%) and 1,8-cineole (19.6%) and Talebi, et al. [22] found 1,8-cineol (43.4%,) as a major component of *N. binaludensis.*

Most of mentioned reports indicate that the major constituents of EO of *N. Binaluednsis* are more or less the same in a different area, but the concentrations of EOs may be varied in different habitats, depending on genetic factors and their growing habitat. For other *Nepeta* species, Amirmohammadi et al. [2] obtained relatively high nepetalactone (73.9% and 55.5) as the main constituents *N. cataria* and *N. assurgens*, respectively. Similarly, Srivastava et al. [21] and Ashrafi et al. [5] obtained high nepetalactone (57.3 and 53.8) in *N. cataria,* respectively.

For other medicinal plants there are different relationships between altitude and EO content. The negative relationships between altitude and EO were obtained for *Nepeta nuda* L. [14], *Origanum vulgare* ssp. hirtum [24], *Thymus migricus* [8] [25], *Satureja bachtiarica* [13], *T. kotschyanus* [10]. In contrast, some positive correlation between altitude and EO content was reported in *N. pogonosperma* (Layegh Haghigi et al*.* [15]), *T. vulgaris* [23] and *T. daenensis* Celak [7].

For EO compounds the trends were partly similar as the EO yield. Some reports indicating negative relationships between altitude and some oil components, like carvacrol in *Coridothymus capitatus* and *Satureja thymbra* [12] and 1,8-cineole in *T. vulgaris* [23]. In contrast, there were positive relationships between altitude and some other EO constituents, such as thymol in *C. capitatus* and *S. thymbra* [12], linalool in *T. vulgaris* [23]) and *T. kotschyanus* [10].

The assessment of EO composition related to environmental conditions can provide important insight into the factors that determine chemical polymorphism in EO. Therefore, the aim of this study was to identify the distribution areas, and evaluate the EO variation and effects of climatic conditions on the EO yield and composition of 20 populations of *N. binaludensis*, as an endemic aromatic and medicinal plant in Iran, to introduce the best germplasm to domestication and improve breeding varieties.

Materials and Methods

The germplasm used in this study included 19 wild populations of *N. binaludensis,* collected from the elevations of Binalud Mountain and one cultivated population in the field of Mashhad's Agricultural Research Center, Iran (altitude 1100 m). The samples of flowering shoots of all populations were collected in the range of 2100 to 2600 m altitude in early July to late August, 2018 (Fig. 1). The localities were Gerineh, Dizabad, Shandiz, Shirabad, Ferizi, Galamkan, Dowlat abad, Kang, Dehbar, kordine, Jaghargh, Feleske, Azghad, Moghan1 and Moghan2. Geographical coordinates of these localities climatic conditions are shown in Table 1.

For each locality, three replications were collected. The plant materials were dried in shade. Then, 60–80 g of the dried flowering shoots of each sample was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The EOs analysed using gas chromatography (GC) and gas chromatography/mass spectrometry (GC–MS). GC analysis was performed using a Thermo-UFM gas chromatograph, equipped with Ph-5 fused silica column (10 m × 0.1 mm i.d., film thickness 0.40 μm). Then

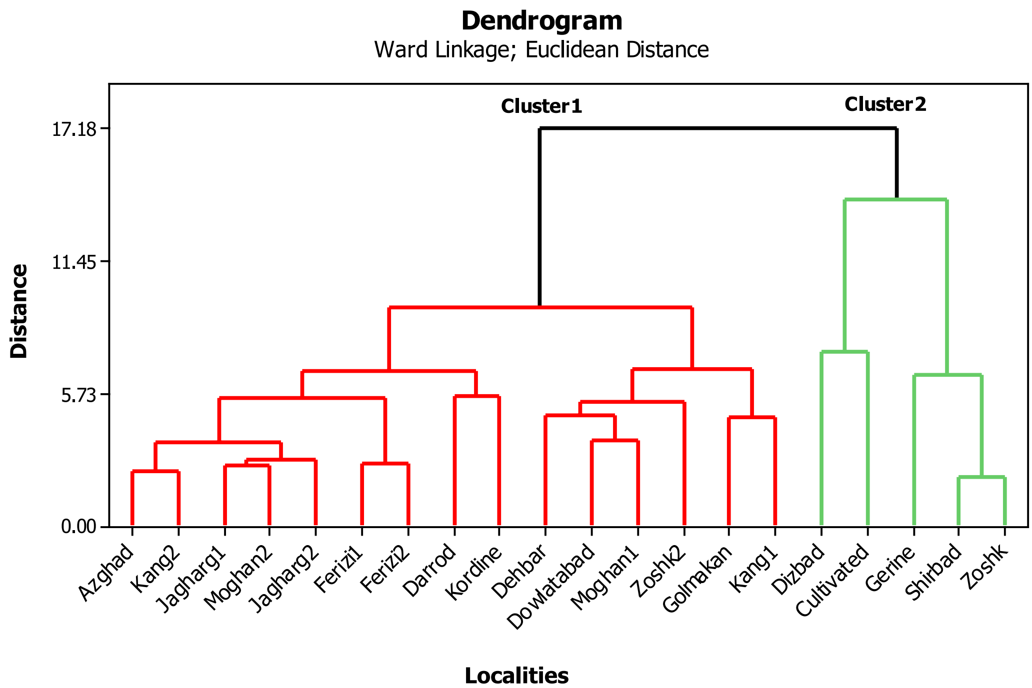

Fig. 1 Picture of *Nepeta binaludensis* in two habitats Binalud mountain, iran

a Varian 3400 GC–MS system equipped with DB-5 column, 30 m in length, 0.25 mm in diameter and 0.25 µm in the thickness were used for EO identification, [1] and [20]. The data of EO yield were statistically analyzed using one-way ANOVA. Mean comparisons were made using Tukey's test at $p \leq 0.05$ levels. Since the EO components were measured in only one replication, therefore, the overall means and standard error of all EO constituents were computed over 20 populations. Relationships among climatic conditions with EO components were determined using correlation analysis. Data were also subjected to principle component analysis (PCA) and cluster analysis (Ward method). The Minitab16 software was used for all of the analysis.

Results

Means of localities for EO yield and oil compound

There was significant difference among localities for EO yields ($p < 0.05$). The highest and lowest EO yield with average values of 4.9% and 1.2% were obtained in Darood and Friezy localities, respectively. The localities of Dowlatabad, Jagharg2, Jagharg1, Moghan2 and Darrod with average values of 3.3, 3.7, 3.8, 4.2 and 4.9%, respectively, had the higher EO yields than the other localities.

Totally, 22 compounds were identified using GC and GC–MC (Table 2). The most substantial constituents in the EO were 1,8-cineol (25.4–59.2%, mean of 42.8%), nepetalactone (13.8–55.1%, mean of 32.7%), myrcene (2.3–5.5%), α-terpineol (2.0–3.6%), trans-sabinene hydrate (0.1–4.4%) and p-cymene (1.1–5.7%).

Correlation between climatic factors and oil compound

Result of correlation analysis showed that EO yield was positively correlated with precipitation and negatively with temperature ($p < 0.05$) (Table 3). The higher value of EO was obtained in high altitude, cold and rainy areas and its value decreased with increasing habitat temperature. Imedicating that domestication of this species in the lowland areas with high temperature may lead to decrease its EO yield. Based on our results, the highest EO yield, was obtained in altitude 2480 m.

Result of correlation analysis showed that many EO compound such as α-pinene, sabinene, myrcene, p-cymene, 1,8-cineole and δ-tepineol were positively correlated with altitude, indicating that the percentage of these compounds increased at higher altitude (Table 3). In contrast, Nepetalactone was negatively correlated with altitude.

Both p-cymene and Tepinen-4-ol were negatively correlated with precipitation and positively correlated with temperature. γ-terpinene, α-pinene and myrcene were

Table 1 Geographical locations and climatic conditions of 20 sampling sites of *Nepeta binaludensis* in Binalud Mountain, Mashad, Iran

No	Local	Altitude (m a sl)	Latitude N	Longitude E	Annual precipitation mm	Hot season raining mm	Wet season temperature °C	Annual temperature °C	Slope degree	Slope %
1	Azghad	2288	36°10′35″	59°16′02″	308	18	5.30	6.20	11.76	20.83
2	Darrod	2480	36°11′07″	59°12′10″	325	22	3.90	4.60	14.47	25.80
3	Dehbar	2413	36°13′19″	59°14′12″	306	18	5.60	6.50	24.70	46.00
4	Dizbad	2457	36°07′43″	59°15′51″	271	11	9.30	10.50	4.28	7.49
5	Dowlatabad	2344	36°18′22″	59°05′02″	301	18	5.70	6.70	25.88	48.52
6	Ferezi1	2391	36°25′30″	58°54′55″	298	19	6.00	6.90	16.81	30.22
7	Ferezi2	2268	36°25′35″	58°54′13″	300	19	5.60	6.50	7.42	13.02
8	Gerene	2630	36°10′35″	59°13′29″	313	19	4.80	5.70	29.13	51.36
9	Golmakan	2375	36°20′03″	59°03′17″	308	20	5.20	6.00	4.30	7.52
10	Jaghargh1	2366	36°18′22″	59°11′08″	303	18	5.70	6.70	20.84	38.06
11	Jaghargh2	2258	36°12′57″	59°12′01″	301	18	5.90	6.90	10.63	18.76
12	Kang1	2575	36°14′40″	59°10′21″	315	20	4.60	5.40	18.57	33.60
13	Kang2	2521	36°14′44″	59°10′24″	320	21	4.30	5.10	10.56	18.64
14	Kordine	2154	36°13′06″	59°12′58″	292	15	6.40	7.50	16.92	30.41
15	Moghan1	2501	36°08′07″	59°18′36″	312	18	5.10	6.00	23.81	44.12
16	Moghan2	2482	36°09′12″	59°16′50″	314	19	4.90	5.70	18.08	32.65
17	Shirbad	2490	36°16′07″	59°07′19″	315	20	4.50	5.40	18.50	33.45
18	Zoshk1	2509	36°15′54″	59°09′09″	323	22	3.90	4.70	19.93	36.26
19	Zoshk2	2650	36°15′50″	59°09′07″	323	22	3.90	4.70	16.37	29.37
20	Cultivated[a]	–	–	–	254	6	7.90	12.50	0.26	0.46

[a] There was no data for origin and of the accession

Table 2 Essential oil composition of *Nepeta binaludensis* aerial parts in flowering stage from 20 sampling sites in Binalud Mountain, Mashhad, Iran

Oil compound	RI[a]	RI[b] adams	Azghad	Darrod	Dehbar	Dizbad	Dowlatabad	Ferizi1	Ferezi2	Golmakan	Gerine	Jagharg1	Jagharg2	Kang1	Kang2	Kordine	Moghan1	Moghan2	Shirbad	Zoshk1	Zoshk2	Cultivated
α-thujene	928	930	0.4	0.5	1.1	0.9	0.4	0.6	0.7	0.6	1.0	0.6	0.5	0.5	0.4	0.5	0.8	0.4	0.6	0.7	0.4	–
α-pinene	939	939	1.0	0.8	2.0	1.6	1.2	1.2	1.3	1.7	1.9	1.2	1.0	1.2	1.0	0.9	1.4	1.1	1.3	1.7	1.2	1.2
sabinene	975	975	0.9	0.7	1.4	0.9	1.4	0.9	0.9	1.9	1.4	1.1	0.8	1.2	1.1	0.7	1.1	1.0	1.2	1.3	1.1	1.4
β-pinene	978	979	0.5	0.4	0.9	0.7	0.8	0.6	0.6	0.1	1.1	0.7	0.5	0.7	0.6	0.5	0.7	0.6	0.7	0.9	0.7	0.8
myrcene	1000	998	3.2	2.3	5.0	4.6	3.7	3.5	3.6	4.9	5.5	3.4	2.9	3.7	3.2	2.5	4.0	3.3	4.4	5.0	3.5	3.8
α-terpinene	1018	1017	1.2	1.0	1.6	0.9	1.4	1.1	1.4	1.2	1.0	1.4	1.3	1.0	1.3	1.3	1.6	1.2	0.9	0.8	2.2	1.0
p-cymene	1026	1024	1.6	1.7	2.0	5.7	1.6	3.0	2.2	2.0	4.4	1.8	2.2	3.8	1.5	1.4	2.5	1.1	3.8	3.6	1.5	2.7
limonene	1029	1029	0.5	0.4	0.8	0.6	0.6	0.6	0.6	0.7	0.6	0.5	0.4	0.5	0.5	0.5	0.6	0.5	0.8	0.8	0.5	0.6
β-phellandrene	1030	1029	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.4	0.3	0.2	0.2	0.5	0.3
1,8-cineole	1031	1031	**38.2**	**25.4**	**41.8**	**55.0**	**45.5**	**34.0**	**32.6**	**52.7**	**56.1**	**42.1**	**33.3**	**50.4**	**40.7**	**29.8**	**45.3**	**32.1**	**59.0**	**55.9**	**34.2**	**52.0**
γ-terpinene	1062	1059	0.8	0.8	1.9	0.9	0.9	0.7	1.3	0.9	1.7	1.2	1.0	0.2	0.8	1.4	1.6	1.1	1.0	1.2	1.3	1.2
terpinolene	1087	1088	0.7	1.1	0.9	0.4	0.8	0.8	0.4	0.7	0.5	0.8	0.9	0.8	1.0	0.9	0.4	0.9	0.8	0.7	0.5	0.4
linalool	1099	1096	0.2	1.9	0.2	0.3	0.2	0.1	0.2	0.2	0.3	0.2	0.2	0.2	0.1	1.9	0.2	0.2	0.2	0.2	0.2	0.7
trans-sabinene-hydr	1101	1098	4.1	0.1	2.1	2.9	2.5	3.9	3.6	2.3	2.7	2.0	3.7	1.9	2.6	–	4.4	2.9	3.0	3.9	1.6	–
δ-tepineol	1168	1166	1.3	1.1	0.9	1.7	1.5	1.3	1.3	1.3	1.3	1.1	1.1	1.4	1.2	0.9	1.2	1.3	1.8	1.7	1.5	1.4
tepinene-4-ol	1179	1177	1.2	0.9	1.2	2.6	1.3	1.9	2.0	1.2	2.1	1.2	1.4	1.2	0.9	1.1	1.8	1.1	1.8	1.9	1.6	1.3
α-terpineol	1190	1188	2.5	2.3	2.0	3.3	2.7	2.8	2.7	2.4	2.5	2.1	2.2	2.1	2.3	2.1	2.5	2.6	3.6	3.4	2.8	2.3
verbenone	1208	1205	0.8	1.0	0.0	0.6	0.4	0.5	0.5	0.3	0.4	0.3	0.8	0.5	0.3	0.7	0.5	0.4	0.4	0.3	1.0	0.6
4aα,7α,7aα-nepetalactone	1364	1360	**39.1**	**55.1**	**32.4**	**14.8**	**31.5**	**40.6**	**42.1**	**21.9**	**14.5**	**36.5**	**43.6**	**22.3**	**38.6**	**50.4**	**26.8**	**46.1**	**13.8**	**14.4**	**42.0**	**26.7**
E-caryophyl-lene	1424	1419	0.4	0.6	0.3	–	0.2	0.2	0.2	0.2	–	0.4	0.3	0.1	0.3	0.5	0.4	0.4	–	–	0.2	0.3
δ-elemene	1439	1438	–	0.7	0.2	–	0.3	0.7	0.7	–	–	0.2	0.5	–	0.3	0.5	–	0.6	–	–	0.5	0.3
cis-β-farnesene	1445	1442	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–	0.2	-
Total com-pounds %			98.9	99.1	99.0	98.7	99.2	99.5	99.2	97.4	99.3	99.1	98.9	93.9	99.0	98.8	98.2	99.2	99.3	98.6	99.2	99.0
Oil yield %			2.1d	4.9a	2.3 cd	1.3e	3.3bc	1.2e	2.1d	2.8c	2.0d	3.8b	3.7b	2.7c	2.7c	1.4e	2.2d	4.2ab	2.9c	2.7c	2.0d	1.8d

The means oil yield (in the last row) followed by the same letters has no significant differences at 5% probability by Tukey test

[a] RI = Retention indices on DB-5 column

[b] RI = Retention indices given in literature [1]

The bold numbers are the two main components of the essential oil

Table 3 Correlations among climatic conditions with essential oil yield and composition of *Nepeta binaludensis*

Essential oil compounds	Altitude m asl	Annual precipitation	Hot season raining	Wet season temp °C	Annual temp °C	Slope degree	Slope %
α-pinene	0.33	− 0.10	− 0.11	0.14	0.13	0.33	0.37
sabinene	0.32	0.23	0.26	− 0.23	− 0.23	0.25	0.27
myrcene	**0.42**[a]	− 0.03	− 0.05	0.07	0.07	0.33	0.37
α-terpinene	0.01	0.12	0.12	− 0.14	− 0.13	0.05	0.03
p-cymene	**0.41**[a]	− 0.34	− 0.35	0.39	0.39	0.10	0.13
1,8-cineole	**0.42**[a]	− 0.04	− 0.10	0.08	0.09	0.23	0.26
γ-terpinene	− 0.01	− 0.01	− 0.12	0.01	0.02	**0.48**[a]	**0.49**[a]
trans-sabinene hydrate	0.00	− 0.09	− 0.05	0.10	0.10	− 0.04	− 0.03
δ-tepineol	**0.44**[a]	0.03	0.06	0.00	0.00	− 0.09	− 0.08
Terpinen-4-ol	0.22	− **0.43**[a]	− 0.38	**0.45**[a]	**0.45**[a]	0.05	0.09
α-terpineol	0.27	− 0.06	− 0.01	0.08	0.08	− 0.11	− 0.10
nepetalactone	− **0.44**[a]	0.07	0.12	− 0.11	− 0.12	− 0.24	− 0.27
Oil yield	0.05	**0.47**[a]	**0.43**[a]	− **0.44**[a]	− **0.44**[a]	0.03	0.01

[a] = significant at 5% probability levels

positively correlated with slope%, indicating that the higher values of this compound could be obtained in steep areas (Table 3).

Cluster analysis,

Cluster analysis, based on EO components, categorized the 20 localities in two groups (Fig. 1 and Table 4). Fifteen localities of (Azghad, Dehbar, Dowlatabad, Darrod, Ferizi1, Ferezi2, Golmakan, Kordine, Kang1, Kang2, Jagharg1, Jagharg2, Moghan1, Moghan2 and Zoshk2) were placed in cluster 1 and five localities (Dizbad, Gerine, Cultivated, Shirbad and Zoshk1) in cluster 2. The localities in cluster 2 had higher mean values of altitude with ranged from 2457 to 2630 m (Table 1). In comparison between clusters, the mean values of 1,8-cineole (38.5 and 55.6%) and the mean values of nepetalactone (37.9 and 16.8%) were obtained in clusters 1 and 2, respectively (Table 4), indicating that clusters 2 with higher altitude had higher 1,8-cineole and lower nepetalactone that was in agreement with correlation between both components with altitude in Table 3.

Principle component analysis (PCA)

The first four main components accounted for 81% of the total observed variation. Results showed the, 8-cineole, myrcene, p-cymene positively and nepetalactone were negatively correlated with PC1. The climatic factors as: temperature, precipitation, altitude and slope correlated with PC2. The compounds of δ-tepineol, γ-terpinene and α-terpinene correlated with PC3 and finally the compounds of tepinene-4-ol, trans-sabinene hydrate, sabinene and EO yield correlated with PC4 (Table 5).

The 20 localities of *N. binaludensis* for were scattered on PCA1 and PCA 2 (Fig. 2 and Table 5). The first component well separated localities similar to cluster separation. The localities in the right side of the diagram had higher values of 1, 8-cineole, myrcene and p-cymene. The localities in the left side had higher value of nepetalactone. The second component was associated with climatic condition and localities in upper side grown in high temperature area. For example in cluster 2 the localities of Dizbad and Cultivated were grown in warmer area coupled with low precipitation and localities of Gerine, Shirbad and Zoshk in lower side of the diagram are grown in cold temperature coupled with high precipitation (Fig. 2). This result indicated that the distribution of localities based on the first two component scores were in agreement with cluster analysis (Figs. 2, 3).

Discussion

There was significant difference among localities for EO yields ($p < 0.05$). The seven localities with range of 2.8 to 4.9% had the higher EO yields than the other localities. These values were much higher than the oil yield (0.8%) that reported by Rustaiyan and Nadji [19] for *N. binaluednsis*.

EO yield was positively correlated with precipitation, and negatively with temperature and the higher EO yields were obtained in high altitude, cold and rainy areas and its value decreased with increasing habitat temperature. However, this trend was not similar in all localities, and there were some exceptions. The published data for some Lamiaceae species species were also different. Layegh Haghighi et al. [15] in *N.*

Table 4 Means of essential oil compound of *Nepeta binaludensis* in clusters 1 and 2 and mean of 20 localities in Binalud Mountain, Mashad, Iran

Oil compound	Cluster 1 (%)	Cluster 2 (%)	Overall means
α-thujene	0.6	0.8	0.6 ± 0.05
α-pinene	1.2	1.5	1.3 ± 0.07
sabinene	1.1	1.2	1.1 ± 0.06
β-pinene	0.6	0.8	0.7 ± 0.04
myrcene	3.5	4.7	3.8 ± 0.18
α-terpinene	1.3	0.9	1.2 ± 0.07
p-cymene	2.0	4.0	2.5 ± 0.26
limonene	0.5	0.7	0.6 ± 0.03
β-phellandrene	0.3	0.3	0.3 ± 0.01
1,8-cineole	**38.5**	**55.6**	**42.8 ± 2.2**
γ-terpinene	1.1	1.2	1.1 ± 0.08
terpinolene	0.8	0.6	0.7 ± 0.05
linalool	0.4	0.3	0.4 ± 0.11
trans-sabinene-hydr	2.7	3.1	2.8 ± 0.24
δ-tepineol	1.2	1.6	1.3 ± 0.05
tepinene-4-ol	1.3	1.9	1.5 ± 0.10
α-terpineol	2.4	3.0	2.6 ± 0.10
verbenone	0.5	0.5	0.5 ± 0.05
nepetalactone	**37.9**	**16.8**	**32.7 ± 2.7**
E-caryophyllene	0.3	0.3	0.3 ± 0.03
δ-elemene	0.5	0.3	0.5 ± 0.05
cis-β-farnesene	0.2	–	0.2 ± 0.00
Total %	98.6	99.0	99.0.40
Oil Yield %	2.8	2.1	2.6 ± 0.22

The bold numbers are the two main components of the essential oil

Table 5 Matrix of coefficients of Eigenvectors, eigenvalues and variance of the first four principal components extracted from main essential oil compositions and climatic factors in 20 populations of *Nepeta binaludensis*

Variable	PC1	PC2	PC3	PC4
1,8-cineole	**0.34**	− 0.10	0.00	− 0.28
myrcene	**0.32**	− 0.19	− 0.18	− 0.11
p-cymene	**0.33**	− 0.05	0.14	0.09
nepetalactone	− **0.36**	0.13	− 0.01	0.19
Wet Season temperature °C	0.20	**0.33**	− 0.07	0.17
Annual temperature °C	0.19	**0.36**	-0.10	0.03
Annual precipitation (mm)	− 0.18	− **0.37**	0.10	− 0.06
Hot season raining (mm)	− 0.18	− **0.36**	0.14	− 0.02
Altitude	− 0.06	− **0.36**	0.12	0.19
Degree of slope	− 0.01	− **0.31**	-0.27	0.12
Slope%	0.01	− **0.30**	-0.28	0.11
δ-tepineol	0.25	− 0.08	**0.37**	− 0.06
γ-terpinene	0.05	− 0.06	**-0.48**	0.25
α-terpinene	− 0.17	− 0.04	**-0.31**	0.23
Tepinene-4-ol	0.30	− 0.07	0.10	**0.42**
trans-sabinene hydrate	0.10	− 0.19	0.17	**0.30**
sabinene	0.18	− 0.12	− 0.23	− **0.49**
Oil yield	− 0.21	− 0.10	0.12	− **0.34**
Eigenvalue	6.38	5.40	2.62	1.78
Proportion	0.32	0.27	0.13	0.09
Cumulative	0.32	0.59	0.72	0.81

[a] The underline coefficients have significant correlation with the relevant axes

By increasing the altitude, changes in growth and essential oil composition of the plant are observed

Result of correlation analysis showed that essential oil yield was positively correlated with precipitation, and negatively with temperature. In the present study the populations with high-amount of nepetalactone and 1,8 cineole were identified,

The populations of cluster 2 with higher amount of 1,8-cineole, were growing in the high altitude, cold, rainy and sloping areas

pogonosperma, Torras et al. [23] in *Thymus vulgaris*, Ghasemi Pirbalouti et al. [7] in both *T. vulgaris* and *T. daenensis* found positive correlation between altitude and EO yield, indicating that domestication of this species in the lowland areas with high temperature may lead to decrease its EO content. Similarly, we obtained the lower EO value (1.8%) from a population that was cultivated in the field in low altitude in Mashad (1100 m) that the value was much lower than mention localities. In contrast, Figueiredo et al. [6] in *T. vulgaris*, Yavari et al. [25] in *T. migricus*, Habibi et al. [10] in *T. kotschyanus* found negative correlation between altitude and EO yield *[10]*.

In EO analysis, totally, 22 compounds were identified, The most important constituents in the EO were 1,8-cineol (25.4–59.2%), nepetalactone (13.8–55.1%), These range of differences were related to genetic variation and climatic conditions of 20 localities. Similar to our result, Rustaiyan and Nadji [19] and Asgarpanah et al. [4] found high value of 1,8-cineol (42.3 and 42.0%), but low amounts of nepetalactone (25.2 and 25.0%) in the EO of *N. binaluednsis*, respectively. Najafi et al. [17] and Mohammadpour et al. [16] found high amounts of 1,8 cineole, 77.8% and 69.3%, respectively, in *N. binaloudensis*. In contrast, Amirmohammadi et al. [2] obtained a high value of nepetalactone (59.7%) but low value of 1,8-cineole (19.6%) in three cultivated *Nepeta* species from Iran. For *N. cataria*, relatively high Nepetalactone (57.3 and 53.8%) were obtained by Srivastava et al. [21] and Ashrafi et al. [5], respectively.

Result of correlation analysis showed that many EO compound such as α-pinene, sabinene, myrcene, p-cymene, 1,8-cineole and δ-tepineol were positively correlated with altitude, indicating that the percentage of these compounds increased at higher altitude (Table 3). In contrast, Nepetalactone was negatively correlated with altitude. Accordingly, Kofidis & Bosabalidis [14], found that altitude significantly affected the chemical

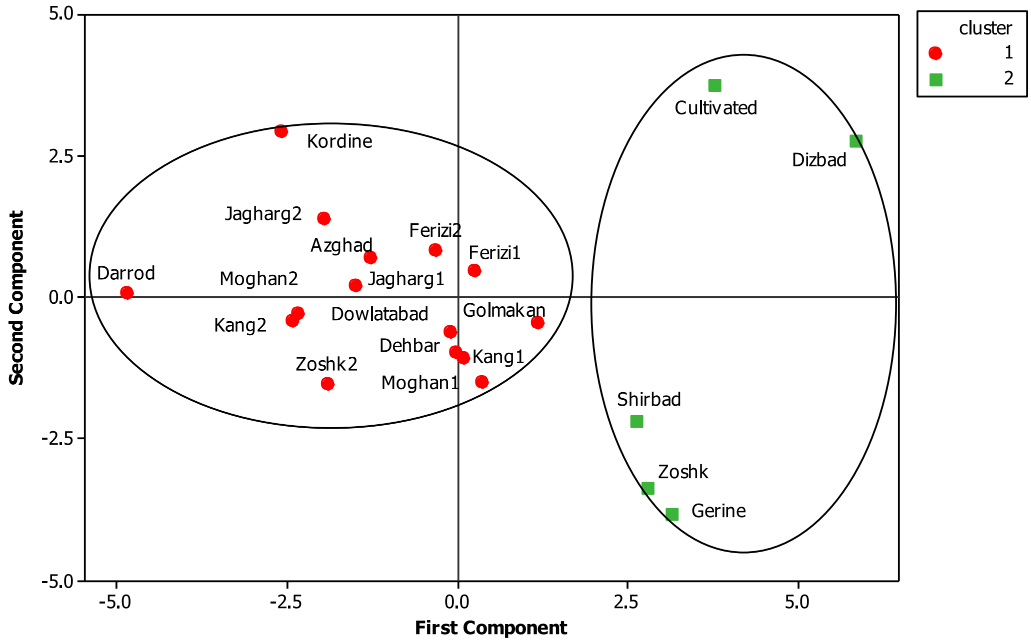

Fig. 2 Dendrogram of the 20 populations of *Nepeta binaludensis* resulting from the cluster analysis of the main essential oil constituents and climatic conditions based on Ward method

Fig. 3 Scatter plot for 20 populations of *Nepeta binaludensis* and three clusters for the first two principal components

composition of essential oil in Lamiaceae taxa. Result of correlation analysis showed that myrcene, p-cymene, 1,8-cineole and δ-tepineol were positively correlated with altitude, indicating that the percentage of these compounds increased at higher altitude. Such positive relationships between altitude and some EO components were reported [10, 12, 15, 23]. In contrast, Aćimović et al. [3] in Serbia reported that temperature positively and precipitation negatively were effective on accumulation of 1,8-cineole in *Nepeta nuda* L. in Rtanj Mountain. It seems their result was obtained in a low land in Serbia.

Nepetalactone was negatively correlated with altitude, indicating that the higher values of this compound could be obtained in low altitude and steep areas.

Result of cluster analysis, showed that nepetalactone was the main component with an average value of 37.9% in the cluster 1, while 1, 8-cineole was the main constituent of cluster 2 with an average value of 55.6%. Results of PCA showed that the first PC was positively correlated with 1, 8-cineole and negatively correlated with nepetalactone. Scatter of 20 localities based on PCA1 and PCA2 showed, in the first axe the localities in the left side had higher values of nepetalactone and localities in the right side had higher values of 1, 8-cineole. This result indicated that the distribution of localities based on the first component is in agreement with cluster and correlation analysis. In this study the localities with high-amount of nepetalactone and 1, 8 cineole were identified, which can be exploited depending on the purpose of breeding and cultivation. The high phytochemical differences in EO composition among *N. binaludensis* localities could provide useful information to improve cultivar with high yield and biological activity.

Conclusion

The localities of Dowlatabad, Jagharg2, Jagharg1, Moghan2 and Darrod with average values of 3.3, 3.7, 3.8, 4.2 and 4.9%, respectively had higher EO than other ones.

The overall means values of 32.7 and 42.8% of were obtained for nepetalactone and 1, 8-cineole, respectively. The localities Gerine, Shirbad and Zoshk had higher value of 1, 8-cineole in the cold area and two localities Dizbad and Cultivated had higher value of 1, 8-cineole in the warm area. For nepetalactone, localities of Ferizi1, Ferezi2, Jagharg2, Moghan2, Zoshk2 and Kordine with a range of 40.6 to 50.4% had higher values than other ones. This variation was related to habitats with different altitudes, slopes and slopes, and coupled with genetic variation. Therefore, in order to introduce the superior cultivar, it is necessary to identify the elite localities and evaluating them in the similar environment in several locations/environments in order to domesticate and introduce high-yielding localities.

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We have no conflict of interest.

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