Journal of Medicinal Plants and By-products (2018) 2: 189-198

Original Article

Ecological Properties of Medicinal Plant of *Hymenocrater calycinus* (Boiss.) Benth. in north- eastern Khorasan, Iran

Ali Mohammad Asaadi[1*] and Asghar Khoshnod Yazdi[1]

[1]*Faculty of Agriculture, Higher Education Complex of Shirvan, Iran.*

Article History: Received: 10 December 2017 /Accepted in revised form: 26 June 2018

Abstract

Hymenocrater calycinus (Boiss.) Benth. is endemic to Iran and it is growing natural habitats in the north east of Iran. In traditional medicine, this plant use for flu treatment, diuretic, antibacterial, antifungal and antioxidant. In order, this plant know ecological characteristic for introduce and dense cultivation on farm land and then use in medical industries. Such as this plant natural sites were controlled of destroying. This study was executed in rangeland of Bojnourd. In the site, the studies were climatically characteristic, stand type, co-dominant plant and vegetable variation, physical and chemical analyses of the soil. The results revealed that this species could be adapted for regions with semiarid- cold climate, annual rainfall average of 332 mm and annual temperature average of 9.82 c°. This species have dispersed in altitude 1400- 2500 m of sea level in mountainous areas. The Soils physical and chemical analyses showed that the soil is very shallow with sandy-loom texture, Ph= 7.79, Ec= 55.97 µs/cm and lime. Principal component analysis (PCA) indicated that the variables of altitude, precipitation, saturation moisture percentage, pH, potassium, calcium and sand had significant correlations with the first axis and explained the 49.84% variation. For the second component, the percentage of organic matter and nitrogen were more important traits and explained the 27.94% variation. The stand type was *Artemisia aucheri- Phlomis cancellata* and the co-dominant plants were over 91 species. Average of canopy covers and density were 3.04% and 1778 shrubs in hectare respectively. The Results indicated that distribution pattern of *H. calycinus* is random in the region.

Keywords: *Hymenocrater calycinus,* Autecology, Rangeland, North Khorasan**.**

Introduction

Since ancient times, plants have been one of the first and most available resources used for treating diseases, and throughout history, there has always been a close relationship between humans and plants, and the herbal medicinal effects and their uses are well known. At present, according to the World Health Organization, up to 80% of the world's people depend on traditional medicine for their basic health needs [1].

The Lamiaceae family is one of the largest and most definite families of flowering plants, with about 236 genera and almost 7170 species worldwide [2]. The family Lamiaceae has a serious function as a resource of medicinal and odorous plants of economical significance. It is rich in secondary metabolites and embodies greatly genera of high economic, medicinal value and essential oils. The genus *Hymenocrater* Fisch. & C.A.Mey. has 24 species in the world [3]; it is observed in Iran, Iraq, Afghanistan, Pakistan, Turkmenistan and Turkey, and nine of them reported in Iran [4,5,6,7]. The genus *Hymenocrater* is named Gol-e-Arvaneh and Dava-e-sheikhali in Persian [3,4]. *Hymenocrater* species has been obtained in Razavi, North and South Khorasan, Azerbaijan, Fars, Golestan, Isfahan, Kermanshah, Kurdistan, Mazandaran, Qazvin, Tehran and Yazd Provinces [8-11]. Plants pertaining to this genus are

*Corresponding author: Department of Plant Production, Higher Education Complex of Shirvan, Iran
Email Address: am-asaadi@um.ac.ir

pharmacologically active and have been utilized in human medicine all around the world [12]. *Hymenocrater* genus wonderfully possesses antioxidant uses as well as antidiabetic, anticoagulant, anti-inflammatory and anticancer activities, due to the presence of notable metabolites including rosmarinic acid and rutin in the aerial branches [13]. *Hymenocrater* calycinus (Boiss.) Benth is endemic to Iran and it is growing natural habitats in the north east of Iran [8].

Natural ecosystems have major functions in the reach of animal food, wildlife habitat, production of industrial and medicinal plants, soil and water conservation in the watershed areas. Vegetation structure is formed based on the effect of various factors (e.g. climate, topography, parent rock and biological agents) on vegetation and soil and based on a certain relationship between these factors in a particular environment. Autecological studies are essential for determination of the ecological necessities of plant species and provide basic knowledge for relevant authorities such as range managers [14] in identifying appropriate plant species for the rehabilitation of degraded rangeland. Plant species are not randomly distributed in the habitat along environmental gradients [15], but display species- specific tolerances shaping non-random assemblages [16]. Among abiotic drivers, climate and soil properties are recognized to strongly constrain the distribution of species [17]. Species are not an independent existence in an ecosystem, but interact ecologically positively and negatively [18].

Mirhoseini *et al* [19] investigated some ecological and morphological factor of *Lagochilus macracanthus* in Yazd Province. They found that this species is distributed in rangelands with 7.7-7.9 pH, 0.13-0.19 EC ds/m in 2300-2450 meters elevation. Akbarlou and Nodehi [20] studied relationship between some environmental factors with distribution of medicinal plants in Ghorkhud protected region. Their results showed that the most important factors affecting the distribution and establishment of dominant species were organic matter, nitrogen, pH, sand and altitude from sea level. Yibing [21] in a study carried out in China using PCA and CA found that soil physical and chemical properties such as nutrients, moisture, salinity, and pH were effective on the homogeneity of habitat. Decent management and optimal utilization of natural ecosystems requires sufficient scientific knowledge and understanding. Therefore, domestication and cultivating domestic plant cultivation can reduce harvest pressure on natural areas for rare, slow grower and critically endangered species [22]. In recent years, reduce rainfall, intensive harvest and land use changes threatened this plant seriously. There are many medicinal herbs in Iran but there is lack of enough information about plants condition and their application. The main purpose of this study was to identify the ecological and phenological characteristics of this species in the natural habitats in North East of Iran.

Material and Methods

Study Area

The region of the study is located in arid rangelands of Bojnourd city in North East of Iran (North Khorasan Provinces). Its geographical coordinates are 37° 18' to 37° 23' North latitude and 57° 8' to 57° 15' East longitudes. The area is approximately 9300 hectares with elevation ranging from 1400 m to 2500 meter. The means of precipitation is 332mm/year that maximum and minimum of precipitation occur in April and September respectively. The mean of annual temperature is 9.82 °C. The average maximum temperature is 31.75.° C in July and minimum temperature is -13.02 °C in February. The climate of this region with using of Emberger method is semi- arid cold. The Embrothermic diagram indicates that drought period is for 125 days of year and wet season start in mid- October and continues until late May (Fig. 1).

Sampling Method

The information of vegetation cover and environmental variables were collected after primary visiting and determining the study area. The climatic (temperature, rainfall), topographic, and edaphic (physical and chemical) characteristics of the habitat were determined. Meteorological data including rainfall and temperature were obtained from Asadli meteorological station. Plant height, density, abundance, canopy cover, the largest and smallest diameter, and yield of *Hymenocrater calycinus* were directly measured in the field using transect and quadrate method.

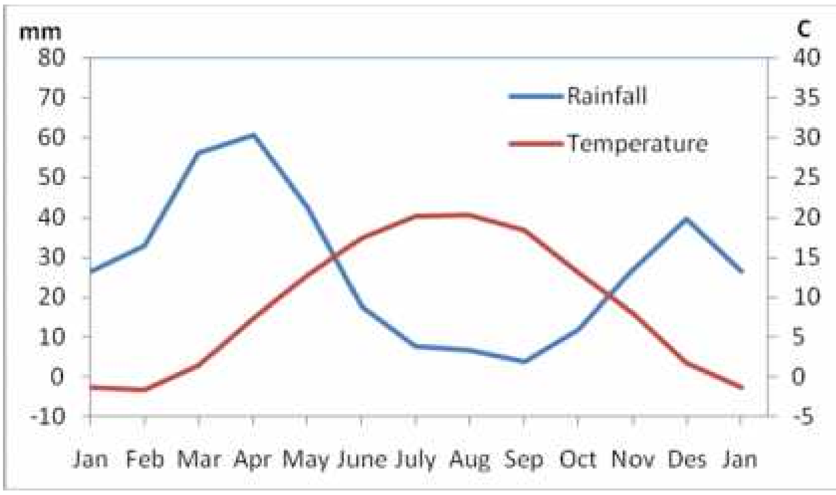

Fig. 1 Embrothermic curve in the study area.

Plot size and sample size were determined by minimum area and vegetation procedure using 80 plots along ten 50 m transects. 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 were recorded in related forms. Rooting depth was also measured in each soil profile. Plant distributions pattern was determined by the Hopkins index:

$$I_h = \frac{\sum(x_i)^2}{\sum(x_i)^2 + \sum(r_i)^2}$$

Where I_h is Hopkins index, x_i is distance of nearest plant and r_i is distance of nearest neighbor plant

Soil samples were carried out with 10 replications from 0-50 cm depth under plant and in control area. Soil samples were analyzed for organic matter percentage, EC, pH, N, Na, K, P, Ca, HCO3, Cl, Mg, SO4, gravel percentage, lime percentage, Saturation moisture percentage and texture. After collecting all data from the sites, analysis was done using SPSS 16 software. T-test were used to compare the data related to planted and control treatments. Data environmental variable were analyzed by multivariate techniques i.e. principal component analysis (PCA).

Results and Discussion

Vegetation survey indicated that *Hymenocrater calycinus* is not the dominant species. *Artemisia aucheri*, *Phlomis cancellata*, *Festuca ovina* and *Bromus inermis* are dominant and associated species in this habitat. This plant grows with several different types of species. Table 1 shows the Co-dominant plant in the habitat.

In table 2, vegetative properties of *Hymenocrater calycinus* (Boiss.) Benth. were presented in the study area. The average canopy cover of *Hymenocrater calycinus* was 3.04% across the habitat. Height of individual plants varies from 22 to90 cm.

Table 1 Some of the Companion species that growing along with *Hymenocrater calycinus* (Boiss.) Benth. in the study areas.

Scientific name	Life form	Scientific name	Life form
Festuca ovina L.	He	*Berberis integerrima* Bunge.	Ph
Ephedra major Host.	Ph	*Berberis khorasanica* Browicz & Zielinski	Ph
Bromus inermis Leyss.	He	*Phlomis cancellata* Bunge	He
Bromus tomentellus Boiss.	He	*Cerasus microcarpa* (C.A . mey) Boiss	Ph
Artemisia aucheri Boiss.	He	*Stipa barbata* Desf.	He
Artemisia kopetdaghensis Krasch.	Ch	*Agropyron trichophorum* (Link) Richter.	He

Ch = Chamaephyte, He = Hemicryptophyte, Ph = Phanerophyte.

The large and small diameters were 16- 123 and10-70 cm. The density of plants in habitat was counted as 1778 per hectare. Product of this plant in areas is 55.12 Kilograms per hectare. This plant has long horizontal and vertical roots.

Table 2 The vegetative characteristics of *Hymenocrater calycinus* (Boiss.) Benth. in the study area

Vegetative characteristics	Unit
Maximum canopy cover	5770(cm^2)
Minimum canopy cover	110(cm^2)
The average canopy cover	1708(cm^2)
The average canopy cover	3.04%
Density	1778(per hectare)
The large diameter Maximum	123(cm)
The large diameter Minimum	16(cm)
The large diameter average	55(cm)
The small diameter Maximum	70(cm)
The small diameter Minimum	10(cm)
The small diameter average	30(cm)
Maximum production per plant	163.2(gr)
Minimum production per plant	3.2(gr)
Mean production per plant	31(gr)
Product per hectare	55.12(kg)
Maximum height	90(cm)
Minimum height	22(cm)
Mean height	45(cm)
Abundance	30%
Spatial pattern (Random)	0.497

These roots can penetrate up to 1 meter depending on soil condition. About 60-70% of the root system was in surface soils (0-40 cm. Distribution of the roots of plants occurs at different soil levels and the form of distribution depends on the type of plant, environmental conditions and type of soil [24]. Spatial pattern of plants can possibly indicate stand history, population dynamics, and species competition. Dispersion or distribution patterns show the spatial relationship between members of a population within a habitat. The distribution of species into clumped, uniform, or random depends on different abiotic and biotic agents [23]. The results showed that distribution pattern of *Hymenocrater calycinus* species are random (Table 2). Random distribution is the most common type of dispersion found in nature [23].With respect to habitat structural characteristics, on the *Hymenocrater calycinus* species situated at the highest altitude (1400-2500 m) The aggregated spatial pattern of the shrub layer prevails, which is caused by extreme conditions of the mountain. This trend of a departure from random distribution is enhanced by vegetative propagation as adaptation to extreme conditions of growth [25,26].

The obtained results of phenological observations in the plant habitat of *Hymenocrater calycinus* shows that with increasing temperature and humidity of the area, its primary buds appear. Vegetative growth stage of this plant starts from late March. With increasing altitude, the vegetative growth stage is delayed so that in the highlands it starts from early-April. Flowering begins from the second week of April and in early May the peak of flowering will occur. Flowering continues to the third week June. After this stage, the plant seeding phase. Seed production started almost from the beginning of early May and continues until the third week of May. The seeds can be separated from the main plant and are scattered around from late June and continues to late August. With the end of summer, plant dormancy period begins and continues until late March (Table 3).

The results of physical and chemical analysis of the soil samples collected from the distribution area of *Hymenocrater calycinus* have been presented in Table 4. The results showed that the soil under this species caused significant increase in soil organic matter, nitrogen and potassium under canopy than outside the canopy. The remainder variables were not significant.

The results indicated that among of Organic matter in the investigated habitat is significantly different. *Hymenocrater calycinus* significantly increased soil organic matter under the plant canopy (Table 4). So that out of the plant shadows is 1.52% and reserves under 2.6%. Mlambo *et al*. in South Africa to study *Colophosprmum mopane* plant amount of organic matter in the canopy obtained from zero to 10 cm depth was significantly higher than that obtained from shading [27]. The increase in organic matter can be attributed to various processes such as accumulation of litter [28], reducing in erosion or increase in sedimentation [29], improve micro-climatic soil conditions [30] or be input sources such as insects, birds and other animals [31]. In general, the amount of organic matter accumulated Litter plant size and there is a direct relationship [32].

Table 3 Phenological stages of *Hymenocrater calycinus* (Boiss.) Benth.

Vegetative stages	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Vegetative growth	- *	* *	* -	- -	-	-	-	-	-	-	-	-
Flowering	- -	- *	* *	* *	-	-	-	-	-	-	-	-
Seed production	- -	- -	* *	* *	*	*	-	-	-	-	-	-
Winter dormancy	* -	- -	- -	- -	-	-	*	*	*	*	*	*

Table 4 The analysis results of soil in the habitat of *Hymenocrater calycinus* (Boiss.) Benth.

Soil properties	Treatment	Average	SE	Sig.
Sand (%)	Under canopy	67.62	2.36	ns
	Outside canopy	58.72	3.12	
Silt (%)	Under canopy	26.28	1.73	ns
	Outside canopy	30.88	0.97	
Clay (%)	Under canopy	6.1	0.96	ns
	Outside canopy	10.40	3.13	
Gravel%	Under canopy	56.23	0.9	ns
	Outside canopy	53.65	5.09	
OM%	Under canopy	2.6	0.197	**
	Outside canopy	1.52	0.075	
PH	Under canopy	7.74	0.034	ns
	Outside canopy	7.79	0.046	
EC (µS/cm)	Under canopy	76.9	10.44	ns
	Outside canopy	55.97	9.55	
Saturation moisture%	Under canopy	54.66	1.25	ns
	Outside canopy	44.04	1.62	
CaCO3%	Under canopy	22.42	0.97	ns
	Outside canopy	25.79	1.62	
N%	Under canopy	0.116	0.075	**
	Outside canopy	0.075	0.003	
P (ppm)	Under canopy	3.86	0.348	ns
	Outside canopy	3.35	0.090	
K (ppm)	Under canopy	24.73	3.01	**
	Outside canopy	9.70	1.29	
Na (ppm)	Under canopy	15.33	2.66	ns
	Outside canopy	13.70	4.47	
CL(mmol/lit)	Under canopy	6.33	0.88	ns
	Outside canopy	6.25	1.03	
$HCO3^-$ (mmol/lit)	Under canopy	3.87	0.74	ns
	Outside canopy	2.75	0.35	
Mg(mmol/lit)	Under canopy	2.93	0.54	ns
	Outside canopy	2.2	0.56	
Ca(mmol/lit)	Under canopy	3.8	1.42	ns
	Outside canopy	1.35	0.22	

Hymenocrater calycinus has a significant impact on canopy soil nitrogen. As can be seen in Table 4, the amount of soil nitrogen was higher under *Hymenocrater calycinus* canopy. Nitrogen levels in the canopy soil and the outside canopy soil was 0.116 and 0.075% (Table 3). The amount of nitrogen fixed by this species is also very significant. Nitrogen is one of the elements that have a significant difference in the soil under the canopy cover of this species. Ghezelseflou *et al.* showed a significant difference in soil N between the canopy and outside canopy of two rangeland Species *Artemisia sieberi* and *Salsola dendroides* [33]. This suggests a significant return of nitrogen through litter. Of course, the presence of microorganisms or fungi that stabilizes N is also possible.

The results revealed that among of Potassium in the researched area is significantly different (Table4). In this investigate, soil K under *Hymenocrater* *calycinus* canopy was significantly higher (24.73 ppm) than outside the canopy (9.7 ppm) (Table 4). Mishra *et al* showed a significant difference in soil K between the canopy and outside canopy of 3 and 6 years old eucalyptus trees [34]. The reason for this increasing could be due to releasing potassium from K-bearing minerals and causes significant increase in K under the canopy of K release or litter decomposition relationships. Banerjee *et al* with the ecosystem studying of which it was the dominant oak species, expressed as the amount of exchangeable potassium in the canopy than outside the shading[35]. Karimian and Razmi, causes an increase in the concentration of potassium in the plant canopy organic matter in the plant shading savings and increased biochemical activity and results in the release of potassium-bearing minerals potassium [36]. Wang *et al* described an increase of organic acids secreted by plant roots leads to release of potassium by plants contain minerals such as potassium feldspar gneiss and is [37]. Tan effect of organic compounds on Humic acid and Folic acid and K release from illite and smectite clays showed [38].

The PCA was carried out to introducing the principal variables from a lot of independent variables. Assisting rate reflects the quantity of original knowledge involved within each factor. 19 Variables were used in the principal component analysis (PCA) in order to ordain the most effective environmental factors controlling the distribution of *Hymenocrater calycinus* habitat. As it is presented in Table 5, the first and second components have accounted for 76.70% of total the variance. The first and second components include 49.7% and 27 % of all variations, respectively. Furthermore, Eigenvectors of environmental factors in relation to axis are shown in Table 6.

According to correlation coefficients between factors and components, the first component involves variables including elevation, Precipitation, Saturation Moisture percentage, pH, Potassium, Calcium and Sand content whereas the second one denotes the percentage of Organic Matter and nitrogen content.

The results indicated that variables such as topography, climatic and soil could affect the existence of this species. Our study indicated that rainfall has a great role in shaping up the vegetation of the mountain area. Generally, according to the obtained results, it seems that precipitation is one climatic indicator for the habitat of *Hymenocrater calycinus* species across the study area. Fatemi Azarkhavarania *et al* in studying the most important climatic factors affecting distribution of *Zygophyllum atriplicoides* in semi-arid region of Iran found out that temperature and precipitation are the main climatic factors affecting distribution of Zygophyllum atriplicoides species in study area [39].

In the present study, *Hymenocrater calycinus* species has a positive correlation with altitude. The altitude is one of the indirect environmental gradients which has direct effects on environmental variables such as climate and soil and directly affect the other factors, including temperature through which plant species distribution will be also changed and the natural habitat structure will be revolutionized. By changing the altitude, precipitation amount and type as well as evaporation and distillation will be varied and thereby, vegetation type will be changed [40]. Mahzooni–Kachapi *et al* indicated that increasing and decreasing the altitude level can change the temperature, relative humidity, wind speed, available water to the plant root and sunlight rates; hence, regarding the altitude level changes, ecophysiological reactions of plant will also change. [41]. Zhang and Dong also documented the altitude is one of the effective factors in the distribution of vegetation types in Lesi plateau of China [42]. The *Hymenocrater calycinus* distribution varied in different aspect of slope. The results indicated that density of *Hymenocrater calycinus* at the various aspects were significantly different (Table 7). This plant density in the southern aspect of slope is lower than the northern, eastern and western slopes. In general in northern hemisphere South aspect time and focus of sun's rays on the surface is longer and comparison with north aspect of slopes. Moisture content is less and production and plant diversity is varying in east, west and north aspects. The slope gradient of the study area is between 20 and 70 percent.

Soil is one of the most important components influencing vegetation. The first axis represents potassium, in the study area; potassium is one of the effective factors in the distribution of habitat. The results of PCA analysis revealed that calcium was an effective factor in the species. Calcium is an essential plant nutrient.

Table 5 The variance of each axis of PCA in the study area.

AXIS	Eigenvalues	Variance (%)	Cum.of Var (%)
PCA1	9.933	49.70	49.70
PCA2	5.394	27.00	76.70
PCA3	2.156	10.80	87.50
PCA4	1.394	7.00	94.50
PCA5	1.122	5.5	100

Table 6 Eigenvector values of the environmental factors in each axis of the PCA technique of the study area

Variable	Eigenvector				
	PCA1	PCA2	PCA3	PCA4	PCA5
Elevation (m)	0.253	0.240	-0.138	0.089	0.018
Clay (%)	-0.221	0.087	0.407	-0.077	0.309-
Silt (%)	-0.212	0.234	0.342-	0.015	0.042
Sand (%)	0.268	-0.170	-0.179	0.055	0.229
Gravel (%)	0.267	-0.148	0.047	-0.131	-0.279
pH	-0.307	0.050	0.100	-0.035	-0.156
EC (µS/cm)	0.263	-0.016	0.297	0.148	-0.287
CaCO3 (%)	-0.064	-0.278	0.069	0.515	-0.379
Organic Matter (%)	0.039	0.413	0.153	0.003	0.102
Saturation Moisture (%)	0.289	0.133	-0.087	0.166	0.128
Nitrogen (%)	0.045	0.410	0.176	0.024	0.083
Potassium (ppm)	0.307	-0.033	0.155	-0.053	0.019
Sodium (ppm)	0.065	-0.282	-0.373	0.060	-0.165
Phosphor (ppm)	-0.204	0.105	0.335	0.420	0.183
Chlorine (mmol/lit)	-0.217	-0.222	0.165	0.075	0.421
HCO3$^-$ (mmol/lit)	0.045	-0.337	0.212	0.012	0.490
Magnesium (mmol/lit)	-0.146	0.097	-0.301	0.622	0.031
Calcium (mmol/lit)	0.299	0.073	0.106	0.200	-0.040
Precipitation (mm)	0.253	0.240	-0.138	0.089	0.018
Aspect of slope	0.270	-0.080	0.297	0.158	0.119

Table 7 Variance analysis for density of *Hymenocrater calycinus* (Boiss.) Benth. in different aspects.

Variable Sources	Sum of Squares	df	Mean Square	F
Density	426003.501	3	142001.167	6.665**
Error	2045328.259	96	21305.503	
Total	2471331.760	99		

Cell wall strength and thickness are increased by calcium addition. Calcium is a critical part of the cell wall that produces strong structural rigidity by forming cross-links within the pectin polysaccharide matrix. With rapid plant growth, the structural integrity of stems that hold flowers and fruit, as well as the quality of the fruit produced, is strongly coupled to calcium availability. Calcium stabilizes cell membranes by connecting various proteins and lipids at membrane surfaces, influences the pH of cells and prevents solute leakage from cytoplasm [43]. Gharavi Manjil *et al* stated that calcium content and acidity are the main factors in classification of plant groups in forests of Guilan [44].

Sand percentage is related to moisture, which is affected by the water retention capacity. Sandy soils have high water retention variance as a capillary effect [45], because finer sand particles have a higher capillary tension head than coarser sand particles. Particle size distribution influences the soil water retention curve, and both fit power-law models.

Analysis of the PCA was showed that the soil moisture has a direct relation with the *Hymenocrater calycinus* species. Jafari *et al* showed that percent of SP is an important and effective factor in ecological species groups in Qom [46]. Available moisture is the most important factor limiting plant growth and distribution of plants. Water availability is the main limiting factor of dry area vegetation [47]. Thus, moisture related variables such as elevation and soil texture are most important for the species composition in arid area [48].

Based on the first component, Soil pH was one of the effective agents in distribution of the *Hymenocrater calycinus* species. The results revealed that this plant is established in the alkaline soil. Acidity directly affects plant growth. The effect of soil pH is great on the solubility of minerals or nutrients. Soil pH is a major factor influencing the availability of elements in the soil for plant uptake [49]. Adel *et al* reported that acidity is one of the most important factors affecting establishment and distribution of *Pinus taeda* species [50]. Virtanen *et al* performed large-scale studies in Eurasia and highlighted the role of acidity in classification of plant species [51].

The second axis represented mainly soil nitrogen and organic matter. Organic matter and nitrogen are vital for plant feeding. Soil organic matter serves multiple functions in the soil, including nutrient retention, water holding capacity, and soil aggregation and is a key indicator of soil fertility. This is in agreement with the results of many other studies that organic matter as an important factor in the distribution of the ground flora [50,52]. Nitrogen being a major food for plants is an essential constituent of protein and chlorophyll present in many major portions of the plant body. Nitrogen plays a most important role in various physiological processes [53].

Conclusion

Generally, each plant species has specific relations with environmental variables. These relations are because of habitat condition, plant ecological needs and tolerance range. *Hymenocrater calycinus* was used in pharmaceutical, forage, and herbal tea in Iran. This plant is endemic to Iran and it is growing natural habitat in the north east of Iran. Results indicated that *Hymenocrater calycinus* generally prefers the climate conditions with low temperature and high rainfall. These results showed that the environmental variables had more effect on morphological characteristics of this species. Soil texture of the region is sandy loam. *Hymenocrater calycinus* grows in the non- saline and alkaline soils and prefers the calcareous soils. The result showed that distribution pattern of this species is random. Hence, we can conclude that at higher altitudes, there are more optimal conditions for this plant domestication and harvest. As climate change has huge effects on endemic species then domestication could be the best way for their protection and conservation.

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