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Original Article

Study on Ethnobotany and the Effect of Ecological Factor on the Yield of Essential Oil of *Ziziphora clinopodioides* Lam. (Case Study: Yazd Province)

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Abstract

Study on relationships between a species with its surrounding biotic and abiotic environment provides valuable information in terms of optimum choice for utilization, propagation, breeding and domestication. In other words, ecological study of behavior as essential elements of their ecosystems and habitat characteristics in order to find appropriate solutions to maintain, revise and revitalize this important part of the renewable natural resources. This study was done to discover the correlation of the essential oil yield of *Ziziphora clinopodioides* Lam. with some environmental factors. The other goal was the ethnobotanical study of the species in different habitats where the plan is growing. The studied areas are located in five natural habitats of Yazd province, Iran. Soil results showed that this species is distributed in rangelands with 7.5-8 pH, 0.633-1.47 EC ds/m. According to 10-year statistics, the average rate of rainfall and annual temperature in these habitats were 175.9-308.7 millimeters and 12.2-17.1 centigrade, respectively. The essential oil showed significant relationship with EC and pH. This relationship was negative but both of them showed significant variance. In other words, the essential oils will increase if EC decrease in a same situation. Investigation on soil texture discovered the ability of the plant to grow in different soil texture. The soil textures in different habitats were silt-loam, sandy-loam, and loamy-sand.

Keywords: Ethnobotany, Biotic Factor, pH, EC

Introduction

Medicinal and aromatic plants are a useful source of primary health care which they used by more than 80% of world's population [1]. One of the most important families for this purpose is Lamiaceae. It is considered as one of the most important plant genetic resources due to very high ecological flexibility to different climates. Due to the existence of various aromatic compounds, they also have many applications in cosmetics and health products [2]. In following, *Ziziphora* L. belongs to the Lamiaceae family. It consists of four species i.e., *Z. clinopodioides*, *Z. capitata*, *Z. tenuior*, and *Z. persica*, which are widely distributed in Iran [3]. *Z. clinopodioides* with the common Persian name "kakuti-e kugi" is a special species that grows wild in Iran, Afghanistan, Iraq and Azerbaijan (Talish). *Z. clinopodioids* is an edible medicinal plant with leaves, flowers and stems that are frequently used as wild vegetable or additive in foods to offer aroma and flavor [4]. The plant is used as stomachic, anti-fever, anti-inflammatory, sedative and flavoring agent in

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Iranian folk medicine. Several studies on *Z. clinopodioides* and its subsp. and ecotypes have shown that the main constituents in the essential oil of this species are pulegone, isomenthone, menthol, menthone, 1,8-cineol, thymol, pcymene, carvacrol, terpinen-4-ol, linalool, piperitone, menth-3-en-8-ol, limonene and *β*-pinene [5-12].

Analysis of plants species and ecological classification of plants is a method for determining of the relationship between vegetation and environment factors [13]. There are many researches about the relationship between habitat characteristics and plant species worldwide [14-20]. Some of the important environmental factors that affect quality and quantity of the active substances of medicinal plants are temperature, light, altitude, water and soil [21]. Ebrahimi *et al* (2011) have found that the low temperature treatment significantly increased biomass, flower yield, essential oil content and plant height of chamomile and significantly difference observed in plants between low and high temperature [22]. According to another research by Goldansaz *et al*. (2017), ecological factor is very important on plant secondary metabolites [23]. Altitude also is another important environmental factor that influences Climatic factors [24]. Nchabeleng *et al*. (2012) found that bush tea harvested from different areas and altitude had different chemical compositions, with bush tea grown in high altitudes contained highest polyphenol content compared to bush tea grown in low altitudes of South Africa. Meanwhile, they showed a positive correlation between altitude and total polyphenol content as compared to the other climatic and soil factors [25].

Ethnobotanical studies provide traditional data for traditional use of natural resources and effective protection of biodiversity [26]. It could be said that, Medicinal plants are a wide variety of species that all or parts of them are used as fresh, dried or processed in order to diagnosis, treatment, prevention, help physiological functions, and maintain the health of humans or animals. Accordingly, most of the countries have invested in production and cultivation of medicinal plants to be used in pharmaceutical, health, cosmetics, and food industries to replace chemical drugs. In Iran, due to the cultural roots, vegetation diversity and richness, and the growth of medicinal plants in different climates and ecological conditions, this issue should be emphasized as a belief. It should be defined as a national necessity within a specific program so that with a scientific knowledge and preservation, improvement, development and proper utilization of medicinal plants, important steps could be taken to improve community health, employment and export. So far, few studies have been conducted on the ethnobotany of medicinal plants in the country, some of which are as follows: Kerman [27], Sirjan [28], Ilam [29], Kashan [30], Kalmand Bahadoran and Bafgh [31], Sistan [32], and Natanz Kashan [33]. In addition, the practice of herbal medicine is accepted in the world of conventional medicine, as clinical research, analysis, and quality control are capable of demonstrating the treatment value of herbal medicine. Traditional medicine is used globally and has a rapidly growing economic importance. World Health Organization also reported that in developing countries, it is often the only accessible and affordable treatment available [34].

The present research aims to investigate the correlation between some environment factors with the quantity of the obtained essential oil from *Z. clinopodioides* in five studied areas. On the other hand, explore about ethnobotanical value of the species, which can help for the cultivation in best situation while get the best essential oil and achieve some valuable information in folk medicine.

Material and Methods

The Selective Site

The study areas are located in five natural habitats in Yazd, Iran. Based on a 10-year report that made by climatically bureau, the average annual precipitation and temperature were determined. The rainfall regime in these areas, as in most parts of the Mediterranean diet, occurs in the cold season. Geographical properties such as altitude, latitude, direction of slop, degree of slop were investigated.

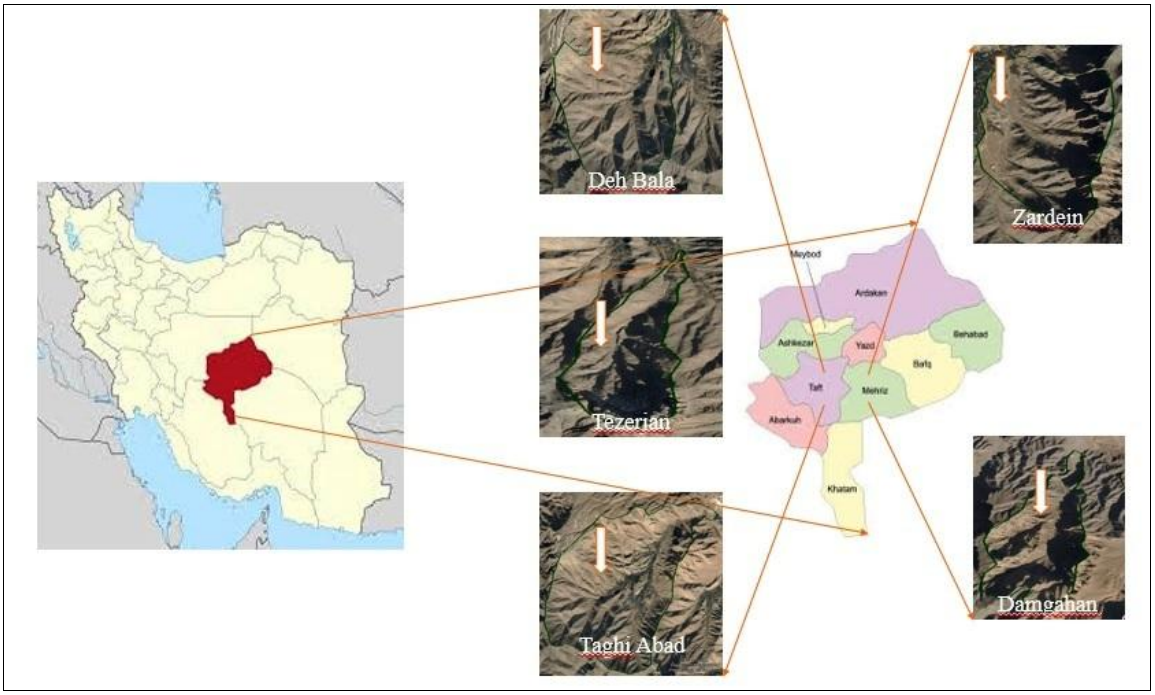

Fig. 1 The studied area

Soil Parameters

Soil samples were carried out with 3 replications from 0-50 cm depth where the root growth is. The samples were passed through a 2mm sieve and subjected to examination some physiochemical factor. The chemical analysis of the soil samples were about nitrogen, phosphorus, potassium and its physical properties such as pH, electrical conductivity, sand, silt, clay and soil texture were measured by Kjeldahl, Titration, Olsen, Flam-photometric, pH meter, EC meter, and Hydrometric method, respectively.

Plant Parameters and Extraction the Essential Oil

The plant materials were collected at each natural habitat with three replications and were dried in the shade at room temperature. Then 50 grams of each community was powdered. After those essential oils were obtained by hydro distillation using a Clevenger apparatus for 3 hours.

Ethnobotanical Investigation

In order to investigate on ethnobotany of *Z. clinopodioides*, 20 villagers in each studied areas were randomly selected who were asked to answer some questions. The questions were about used parts, diseases to be treated, kind of use, and how to use. In order to convert qualitative data to quantitative data, the methods of Shetaye were used.

Statistical Analyses

The soil characteristics and essential oils were analyzed by SAS. Investigation on the correlation between essential oils and some ecological factors were done, too. The results are presented as mean ± standard error. The analysis of variance (ANOVA) and LSD tests were used for statistical analysis and values of $p \leq 0.05$ were considered as significant indicators.

Results and Discussion

The Studied Areas

According to 10-year statistics, mean annual temperature and precipitation were varied between 12.2-17.1 °C and 175.9-308.7 mm, respectively. The rainfall regime in these areas, as in most parts of the Mediterranean diet, occurs in the cold season. There is some similar investigation on this species [35,36]. This genus was investigated in Hamedan and Kordestan, too. The average temperature and rainfall were 8-10.5 °C, 347-430 m (Hamedan) and 11.3 °C, 509 (Kordestan), respectively. The altitude in different studied areas in Yazd was 2414-2791. It could be said that the plant needs to grow in highlands. The suitable altitudes were varied between 1710-2335 (Hamedan) and 1755-1780 m (Kordestan). There are some differences in the present study with the other researches.

Table 1 Different characteristics of the studied areas

Parameter / Habitat	Geographical properties		Topography			Climate	
	Latitude	Longitude	Altitude (m)	Slop %	Slop direction	Temperature (°C)	Rain (mm)
Damgahan	31° 30' 54" N	54° 18' 46" E	2414	49.99	SE	15.3	231.7
Taghi Abad	31° 34' 24" N	54° 07' 14" E	2736	16.14	N	12.2	308.7
Zardein	31° 30' 04" N	54° 14' 17" E	2610	27.04	W	17.1	175.9
Tezerjan	31° 34' 28" N	54° 09' 30" E	2532	55.88	SE	14.8	219.5
Deh bala	31° 34' 53" N	54° 05' 22" E	2791	15.7	NE	12.2	308.7

Soil Results

This plant was more abundant on soils with pH that varied between 7.5-8, and Ec ranged from 0.633 to 1.47 DS/m. This is consistent with the findings reported by similar research that the species can tolerate soil pH 7.9-8.1 [34]. The content of nitrogen, phosphorus and potassium in the soil varied 0.034-0.068, 3.93-6.1, and 58.33-162, respectively. As can be seen in the figure 2, EC in Zardein had significant different with others. pH in Zardein and Tezerjan (7.8 and 8) were higher than Taghi abad, Deh bala, and Damgahan (7.5). The edaphic factor has a high impact on the distribution of plants in desert areas. The soil characteristics play a key role in plant produce. In this regards EC and pH are more significant than the others [37]. These points are completely accordance with the present study. Sand had significant difference in all studied areas that the lowest and highest one belonged to Deh bala and Tezerjan, respectively. The significant variety in sand, silt, and clay caused different soil texture. The soil textures for Taghiabad, Deh bala, Damgahan, Zardein, and Tezerjan were Silt-Loam, Silt-Loam, Sandy-Loam, Sandy-Loam, and Loamy-Sand, respectively. The results of the present survey were different in soil texture [35,36].

Correlation between some Environmental Factors and the Yield of Essential Oil

There is a hypothesis that with a scientific knowledge and preservation, improvement, development, and proper utilization of medicinal plants, important steps could be taken to improve community health, employment and export [38]. For this purpose, one of the most important things in medicinal plant cultivation is soil nutrients. Based on this important point, the correlation between soil nutrients with the essential oil was measured. It should be said that, secondary metabolites like first metabolites are directly affected by environmental factors. EC and pH were more important factors than others among the soil properties [36]. This is in accordance with the present data. As can be seen in table 2, EC and pH showed negative correlation with essential oil. On the other hand, EC and pH have inhibited in 1% and 5% significant difference, respectively. A research group stated that knowledge of species habitat conditions and their classification in ecological species groups is of particular importance [38]. Investigation on environmental factors not only discover the relationship with secondary metabolites, but also determine the relationship between vegetation and environmental factors [39]. There are many researches about the relationship between habitat characteristics and plant species worldwide, which indicate the influence of habitat characteristics on secondary metabolites [40-46]. On the other hand, the ecological system is one of the most common vegetation classification systems. In this system, the species having similar ecological relations with environmental factors are classified in a group as ecological species group. These plant species often have a similar distribution in natural areas. Therefore, the vegetation unites are recognized based on ecological species groups instead of indicator species [46]. Undoubtedly, this category will help for cultivation and use of positive secondary metabolites.

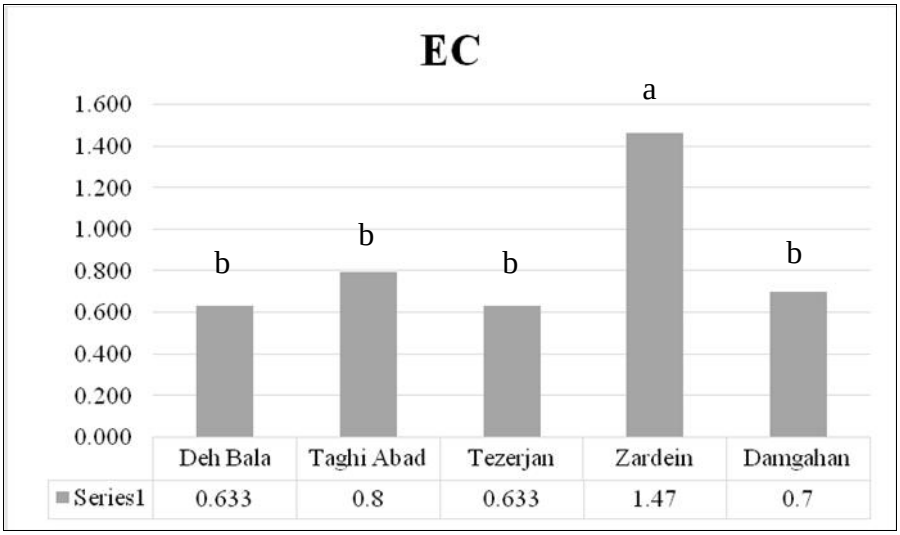

Fig. 1 Average comparison for EC

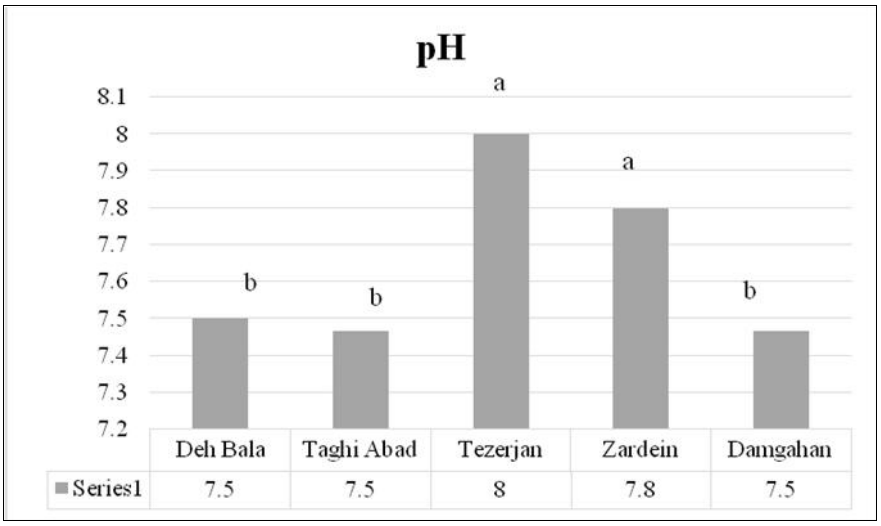

Fig. 2 Average comparison for pH

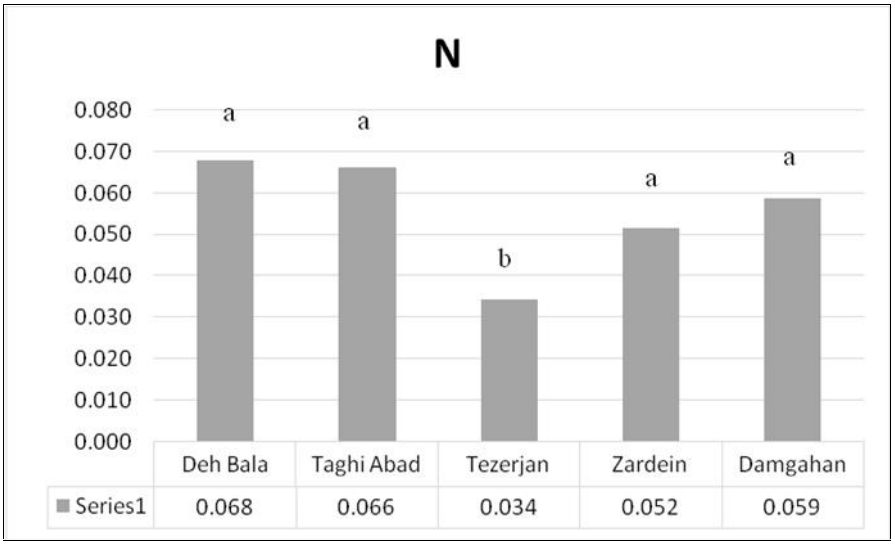

Fig. 3 Average comparison for N

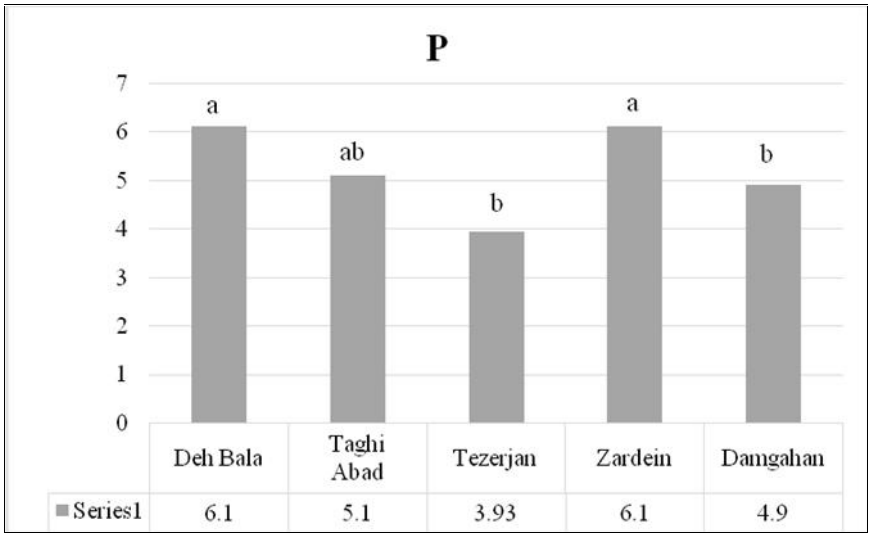

Fig. 4 Average comparison for P

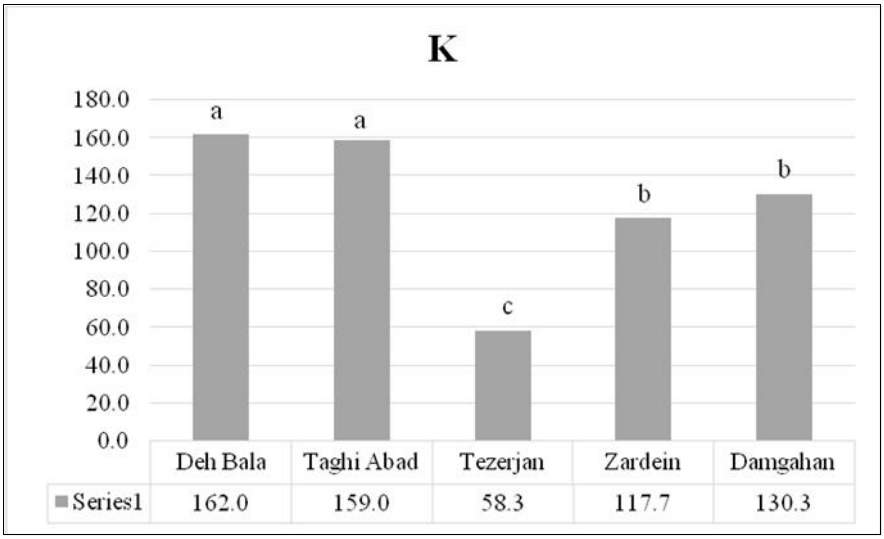

Fig. 5 Average comparison for K

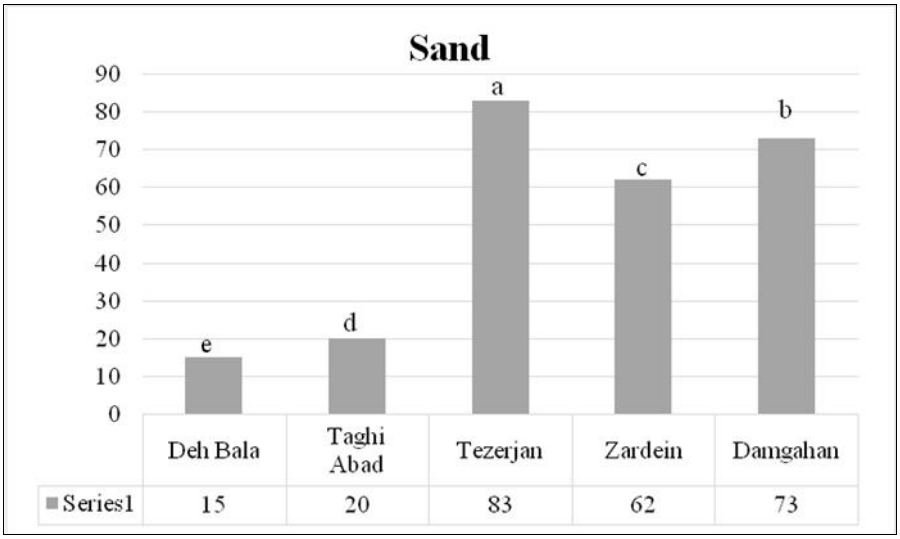

Fig. 6 Average comparison for Sand

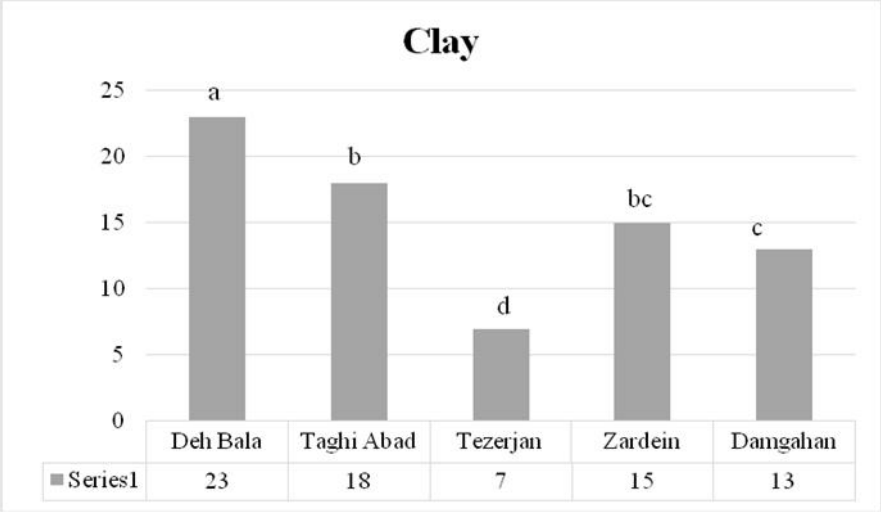

Fig. 7 Average comparison for Clay

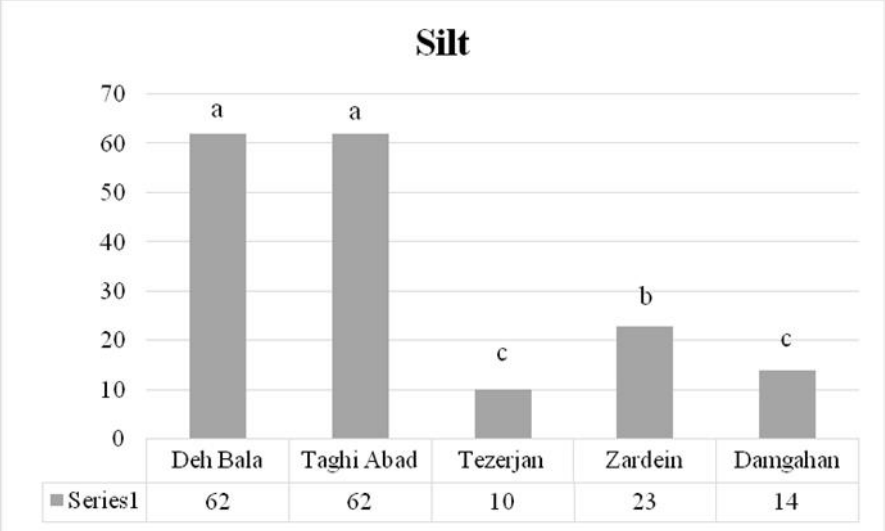

Fig. 8 Average comparison for Silt

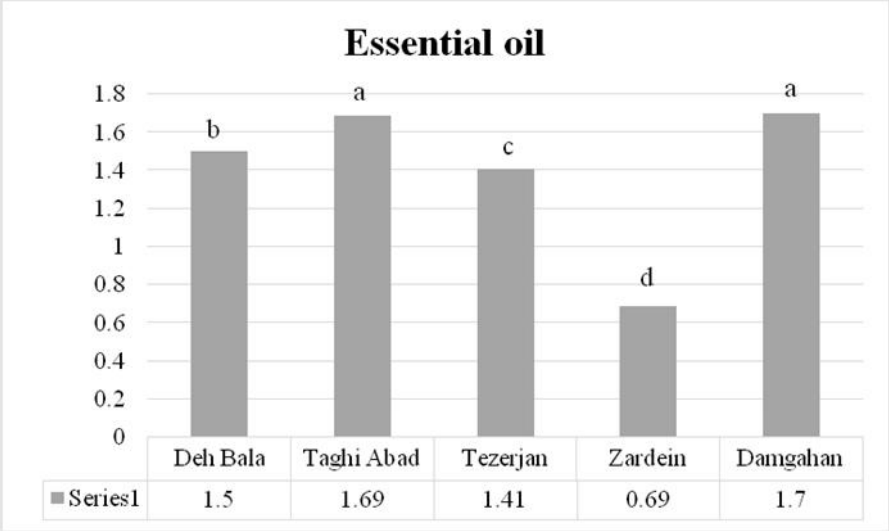

Fig. 9 Average comparison for essential oil of *Ziziphora clinopodioides* Lam.

Table 2 Correlation between investigated factors

Factor	N	P	K	EC	pH	Sand	Clay	Silt	Essential oil
N	1.000	-	-	-	-	-	-	-	-
P	0.476 ^{ns}	1.000	-	-	-	-	-	-	-
K	0.804 **	0.532 *	1.000	-	-	-	-	-	-
EC	0.006 ^{ns}	0.416 ^{ns}	-0.03 ^{ns}	1.000	-	-	-	-	-
pH	-0.719 **	-0.353 ^{ns}	-0.809 **	0.140 ^{ns}	1.000	-	-	-	-
Sand	-0.719 **	-0.498 *	-0.834 **	0.112 ^{ns}	0.611 **	1.000	-	-	-
Clay	0.711 **	0.667 **	0.897 **	-0.005 ^{ns}	-0.677 **	-0.888 **	1.000	-	-
Silt	0.698 **	0.443 ^{ns}	0.793 **	-0.134 ^{ns}	-0.576 *	-0.994 **	0.833 **	1.000	-
Essential oil	0.312 ^{ns}	-0.357 ^{ns}	0.279 ^{ns}	-0.831 **	-0.522 *	-0.259 ^{ns}	0.105 ^{ns}	0.287 ^{ns}	1.000

Table 3 Ethnobotanical values

Regions	Taghi Abad				Damgahan				Zardein				Deh bala				Tezerjan			
Questions	F	FL	RPL	ROP	F	FL	RPL	ROP	F	FL	RPL	ROP	F	FL	RPL	ROP	F	FL	RPL	ROP
Parts used																				
Leaves	9	16	0.1	1.6	3	13.8	0.2	2.8	1	2.2	0.04	0.08	0	0	0	0	1	2.1	0.04	0.08
Flower	12	17.1	0.1	1.7	0	0	0	0	7	17.9	0.39	6.98	18	18.9	0.8	15.1	8	11.4	0.32	3.7
Aerial part	18	66.9	1	66.9	17	86.2	0.8	68.9	15	79.9	0.9	71.9	12	81.1	0.7	56.8	20	86.5	0.8	69.2
Root	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diseases to be treated																				
Cold	3	2.6	0.05	0.13	6	15.9	0.33	5.25	16	31	1	31	5	7.5	0.2	1.5	14	17.1	0.5	8.5
Toothache	11	4.9	0.14	0.7	4	16	0.25	4	7	17.5	0.39	6.98	0	0	0	0	12	17.1	0.5	8.5
Digestive system	20	92.5	1	92.5	20	50.1	1	50.1	20	51.5	1	51.5	20	92.5	1	92.5	20	65.8	1	65.8
ETC	0	0	0	0	7	18	1	18	0	0	0	0	0	0	0	0	0	0	0	0
Kind of use																				
Diseases	20	48.9	1	48.9	12	11.4	1	11.4	16	2.1	0.04	0.08	3	74.6	0.2	14.9	9	66.8	0.35	23.4
Spice	15	35.7	0.7	24.9	13	17.1	0.48	8.2	8	10.3	0.8	8.24	9	14	0.3	4.2	20	19.1	1	19.1
Aromas	7	15.4	0.27	4.16	14	71.5	0.7	50	12	87.6	0.7	61.3	8	11.4	0.3	3.4	15	14.1	0.6	8.49

F: Frequency: All people who answered the questions
FL: Fidelity Level: Percentage of the same answers to the same questions
RPL: Relative Popularity: Degree of general relatively
ROP: Rank Order Priority: FL with corrected coefficient

Ethnobotanical Investigation

Ethnobotanical studies reveal that cultural attitudes and perspectives on the use and application of bio-resources within their communities constitute a valuable component in conservation, bio-prospection, domestication and improvement of plant-based products. However, the continuation of this knowledge is endangered when transmission between older and younger generation is no longer happening [47]. The impetus to document ethnobotanical information of indigenous people should increase as we stand to lose such traditions the world over; including the knowledge of how to recognize and use economically valuable wild plant species [48].For this purpose, *Z. clinopodioides* was studied. The ethnobotanical investigation showed the species had various values. In other words, *Z. clinopodioides* is used in different purposes. Questionnaire in the studied areas revealed that aerial part was used as a main part of used. The species was used as a natural remedy in different references and the present results were accordance with the them [49,50]. The most use in various areas was about digestive system. This kind of research does not only complete previous study but also provide some new information. Table 3 shows all details about ethnobotanical value of the species which was investigated. There is a noteworthy that, the present study is in accordance with previous study. Some researchers believe that documentation of the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources [51]. Therefore, establishment of the local names and indigenous uses of plants has significant potential societal benefits [52]. If a plant is used to treat the same disease in different places across the world then its pharmacologic effect could be accepted. It would be beneficial to conduct pharmacologic studies on such plants. Therefore, it is suggested that such studies may make contributions to indigenous ethnobotanical knowledge as well as studies of the sourcing of raw materials for development of commercial pharmaceuticals [53,54]. On the other hand, some researchers recorded wild useful plants used for different purposes by the local inhabitants in their floristic studies and ethnobotanical works [55-57]. Table 3, showed this remarkable point. In different study area, people used the species for different purpose. Based on the present ethnobotanical investigation, *Z. clinopodioides* is a botanical species well-known in the studied areas and appreciated for its leaves, flower, and aerial parts. Various ethnic groups use the leaves as a source of food (vegetable leaves). They are consumed fresh or reduced to powder. The various organs of the plant are used to help in the cure of various diseases such as Cold, Toothache, Digestive system, etc. All the organs of the species are used to various degrees, and the aerial part is the most used organ.

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