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ECO-PHYSIOLOGY OF ZIZIPHORA CLINOPODIOIDES LAM. (LAMIACEAE) FROM IRAN

Amiri F.[1], Gholipouri A.[1,*], Kheirkhah M.[2], Mirjalili M.H.[3]

[1] *Department of Agronomy and Crop Breeding, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran*
[2] *Department of Plant Production Technology, Faculty of Agriculture, Higher Education Complex of Shirvan, Northern Khorasan, Iran*
[3] *Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C., Tehran, Iran*
**Corresponding author: Telephone: +989143537891; e-mail: gholipouri@uma.ac.ir*

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Abstract - The current research aims to investigate some eco-physiological factors of *Ziziphora clinopodioides* Lam. subsp. *rigida* (Bioss.) Rech. f. in different habitats. Studied parameters included topography, climate, and physiochemical properties of soil. Co-dominant plant, phenology, and vegetation variation were considered, too. In following, the effect of different climatic conditions on some physiological traits of *Z. clinopodioides* were investigated. For this purpose, five *Z. clinopodioides* populations were collected at two phenological stages (vegetative and full flowering). The highest density (0.37) and frequency (23.33%) were registered in Deh Bala and the lowest were in Damgahan and Tezerjan. Based on standard methods pH (7.5-8.0) and EC (0.633-1.470 dc/m) were calculated and the calculated data was different in various habitats. The content of chlorophyll a (9.73–13.18 mg/g), chlorophyll b (2.02–2.88 mg/g), total chlorophyll (11.76–15.98 mg/g), carotenoid (4.48–5.95 mg/g), proline (0.360–0.371 mg/g), and soluble sugar (0.18–0.23 mg/g) varied in the studied species. The phenological stages and climatic conditions significantly affected on the studied pigments except chlorophyll b. The interaction between phenological stages and environmental conditions effected on soluble sugar significantly.

Keywords: pH; EC; Soil texture; Density; Proline; Chlorophyll; Carotenoid.

Introduction

The genus *Ziziphora* L. belongs to the Lamiaceae family. *Z. clinopodioides* Lam, *Z. capitata* L., *Z. tenuior* L., and *Z. persica* Bunge. are wild spread in Iran (Assadi et al., 2012). Nowadays, many researchers are interested to focus on collection, domestication, conservation, and breeding of medicinal and aromatic plants. In following, interest in plant ecology has increased (Abdel-Ghani et al., 2011). Environmental factors affect on species that these effects provide valuable information in terms of optimum choice for utilization, propagation, breeding and domestication. In other words, understanding the ecological requirements of native plants can be useful for applied issues (Azarnivand & Dsamalchi, 1998). Briefly, identifying ecological factors such as soil, topography, and climate that influence plant distribution is essential to conserve, manage and improve rangeland ecosystems (Ajir & Shahmoradi, 2007; Nautiyal et al., 2009).

Environmental conditions impress not only the plant growth but also influence on physiological factors and secondary metabolites. Most plants show a significant variation in active ingredients during different seasons, too. It could be said that the location of growing, harvesting season, and genetics of plant influence these aspects (Mane et al., 2011; Farahbakhsh et al., 2017). In other words, environmental factors such as temperature, humidity, drought and nutrient, which change during seasons and location, can alter the plant physiology and productivity leading to metabolite changes during plant growth and development.

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Based on IPCC (Intergovernmental Panel on Climate Change) reports, climate change is occurring, and it influences all ecosystems. These changes such as drought cause some stresses, which affect the physiological factor. Undoubtedly, plant defence system will be active against stress. Because of interaction between stress factors and plant physiology, resistance of plants to drought stress is very complicated. It could be achieved by the mechanisms of osmotic regulation due to the accumulation of compatible solutions such as proline and carbon hydrates (Hirai et al., 2000; Ajithkumarand et al., 2013). Osmotic protectants are extensively produced in plants to adjust the drought and osmotic stresses. The soluble sugar, proline and the other compounds called osmolytes are the most important osmotic regulators produced under water deficit (Yordanov et al., 2000; Sairam & Tyagi, 2004). Proline and hydroxyproline in structural proteins are clearly distinguished from free proline, which serves to regulate osmotic adjustment (Ueda et al., 2007). Proline accumulation has a significant role in plant adaptation. Proline as a compatible solute plays an essential role in intracellular osmotic regulation, stabilizing the structure of proteins and cell membranes, sweeping the radical oxygen species (ROS), pH adjustment, cellular reaction of oxidation and oxidation reactions (Kavi Kishori et al., 2005; Verbruggen et al., 2008). Contents of photosynthetic pigments, including chlorophylls and carotenoids, which are important in converting light energy to chemical energy, change under various situation (Jaleel et al., 2009). These changes can create limitations in photosynthesis with reducing the concentration of chlorophylls and carotenoids in different stages (Reddy et al., 2004). According to these situations, the variations in different habitats and thus in plant physiology must be monitored throughout different seasons. Some scientists try to find more information about it. The amount of proline and soluble sugar in *Suaeda altissima* Pall. and *Frankenia hirsute* L. in two stages were discovered by Teymouri et al. (2014). Sharifi-Rad et al. (2017) investigated the effects of different harvesting intensities and phenological stages on soluble carbohydrate variabilities of *Halocnemum strobilaceum* and *Halostachys caspica* (Sharifi-Rad et al., 2017). Azari Nasrabad et al. (2017) studied the drought stress effect during different growth stages on yield, osmolites and photosynthetic pigment accumulation of grain sorghum genotypes (*Sorghum bicolor* L.). They stated that proline, soluble carbohydrates, and stem sugar content increased under drought stress and photosynthetic pigments were reduced (Azari Nasrabad et al., 2017). Based on distribution of plant species in various areas, it is much important to understand the ecological requirements. This information provides some valuable knowledge for the application of appropriate plant species in different ecosystem. Although several studies have been published indicating ecological requirements and the effect of phenology on physiological factors, there is no enough information about *Z. clinopodioides* Lam. subsp. *rigida* (Bioss.) Rech. f. According to this purpose, the present study focused on some ecological factors and the effects of phenological variation and different locations on distribution and some physiological factors.

MATERIALS AND METHODS

Study area

Studied areas were located in Yazd, Iran. As can be seen in Table 1, geographic coordinates, topographic features (slope, slope direction, and altitude), and climatic (average temperature and rainfall) were studied separately. The field study was carried out around Shirkouh as a main mountain in central of Iran.

Table 1. a) Geographycal location and b) climate of the study areas.

Parameter	Geographical properties	
Habitat	Latitude	Longitude
Damgahan	31° 30’ 54” N	54° 18’ 46” E
Deh Bala	31° 34’ 53” N	54° 05’ 22” E
Taghi Abad	31° 34’ 24” N	54° 07’ 14” E
Tezerjan	31° 34’ 28” N	54° 09’ 30” E
Zardein	31° 30’ 04” N	54° 14’ 17” E

b)

Parameter	Topography			Climate	
Habitat	Altitude (m)	Slope %	Slope direction	Temperature (°c)	Rain (mm)
Damgahan	2414	49.99	SE	15.3	231.7
Deh Bala	2791	15.7	NE	12.2	308.7
Taghi Abad	2736	16.14	N	12.2	308.7
Tezerjan	2532	55.88	SE	14.8	219.5
Zardein	2610	27.04	W	17.1	175.9

Soil sample

Soil samples were carried out with 3 replications from 0-50 cm depth where the root is growing. The samples were passed through a 2 mm sieve and some physiochemical factor were subjected to examination. 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 hydrometr method, respectively.

Vegetation study

The collected plant was compared with the herbarium specimen and flora Iranica to be approved. In following phenology, co-dominant plant and vegetation variation were determined. In order to investigate vegetation variation, the study areas were divided to three parts by using three transects 50 meters apart about 50 meter from each other parallel to the line level. On each transect 10 points with a distance of 5 meters were considered. According to type of vegetation, 1 m^2 plot were used to evaluate the characteristics of the plant. Based on these plots, density, frequency, average production, and canopy cover (largest length, width and height) were calculated.

Plant materials

The aerial part of five natural *Z. clinopodioides* Lam. subsp. *rigida* (Bioss.) Rech. f. populations (ZCPs) were collected at two phenological stages (vegetative and full flowering) from early April until mid-June.

Physiological characteristics

Soluble sugar, proline, carotenoid, and chlorophyll contents were measured by using standard methods (Troll & Lindsley, 1955; Li, 2000; Lichtenthaler et al., 2001).

In order to investigate soluble sugar content, 70 mg of dried samples were placed in 2 mL of ethanol (80%). The samples were heated at 80 °C for 15 minutes, incubated, and then used centrifuge (5500 rpm) for 10 minutes. This method was done twice for each sample and after completion of the centrifugation; the clear solution was removed from the test tubes. The supernatants were mixed and the volume was upped to 6 ml with ethanol (80%). All samples were cooled and 4 ml of anthrone was added. The mixture was heated at 100 °C for 10 min and absorbance was read at 630 nm (Li, 2000).

According to extracting fresh samples in 3% sulfosalicylic acid, proline was determined. The extracts were heated using a water bath for 10 min and filtered through filter paper. Then two millilitres of the extract were mixed into 6 ml assay media containing 2 ml of ninhydrin solution and 2ml acetic acid. After that, all samples were incubated at 100 °C for 30 min and cooled by room temperature. The coloured product was extracted by adding toluene. Finally, the absorbance of the organic layer was measured at 520 nm (Troll & Lindsley, 1955).

To determinate chlorophyll a, b, and carotenoid content, 0.5 g of fresh leaves were crashed in a pounder. 20 ml of acetone 80% were added to the crashed material. After extraction, the absorbance was read at 645, 663, and 470 nm, respectively. According to the following formula, the exact quantities of the contents were measured (Lichtenthaler et al., 2001).

Chl a (μg/ml) = $12.25A_{663} - 2.798A_{645}$
Chl b (μg/ml) = $21.50A_{645} - 5.10A_{663}$
Chls a+b (μg/ml) = Chl a + Chl b
Carotenoid (μg/ml) = $(1000A_{470}$-1.63Chla-104.96Chlb)/221

Statistical analysis

The data were subjected to SAS 9.4 and analyzed by using ANOVA. Probability levels of 1% and 5% ($P < 0.01$ or 0.05) were used to test the significance among the treatments. The physiological data and soil data were analyzed as a 2×2 factorial with a completely randomized and completely randomized design, respectively. All tests were done with three replications. When an F-test indicated statistical significance, the protected least significant difference (LSD) was used to split the means of main effect. Interaction effects were divided by slicing method.

Results and Discussion

Soil Results

As can be seen in Table 2, the species of interest was abundant on soil with pH that varied between 7.5-8.0, and EC ranged from 0.633 to 1.470 dc/m. This is consistent with the reported findings by similar research that the species can tolerate soil pH 7.9-8.1 (Azimi, 2009). Table 3 shows mean comparisons of chemical characteristics of soil samples, which the calculated nutrient elements were nitrogen (0.034-0.068 %), phosphorus (3.93-6.10 mg/kg) and potassium (58.33-162.00 mg/kg). The soil investigation showed Zardein had significant different with others in soil salinity. pH in Zardein and Tezerjan (7.8 and 8.0) were higher than Taghi Abad, Deh Bala, and Damgahan (7.5). The edaphic factor has a high impact on distribution

Table 2. Mean comparisons of physiological characteristics of soil samples.

Variations / Study areas	pH	EC (ds/m)	Sand (%)	Silt (%)	Clay (%)	Soil texture
Damgahan	7.5 ± 0.11 b	0.7 ± 0.173 b	73 ± 2.65 b	14 ± 3 c	13 ± 2 c	Sandy Loam
Deh Bala	7.5 ± 0.1 b	0.633 ± 0.06 b	15 ± 1 e	62 ± 2 a	23 ± 1 a	Silt Loam
Taghi Abad	7.5 ± 0.06 b	0.8 ± 0.1 b	83 ± 2 a	10 ± 2.65 c	7 ± 1 d	Silt Loam
Tezerjan	8.0 ± 0.0 a	0.633 ± 0.06 b	20 ± 1 d	62 ± 2.65 a	18 ± 2.65 b	Loamy Sand
Zardein	7.8 ± 0.2 a	1.47 ± 0.23 a	62 ± 2 c	23 ± 2.65 b	15 ± 1 bc	Sandy Loam

Table 3. Mean comparisons of chemical characteristics of soil samples.

Variations / Study areas	N (%)	P (mg/kg)	K (mg/kg)
Damgahan	0.059 ± 0.015 a	4.9 ± 0.3 b	130.33 ± 12.66 b
Deh Bala	0.068 ± 0.006 a	6.1 ± 1.11 a	162 ± 9.0 a
Taghi Abad	0.066 ± 0.008 a	5.1 ± 0.44 ab	159 ± 9.16 a
Tezerjan	0.034 ± 0.008 b	3.93 ± 0.45 b	58.33 ± 6.03 c
Zardein	0.052 ± 0.006 a	6.1 ± 0.62 a	117.67 ± 3.51 b

Table 4. Mean of vegetation variation in the study areas.

Variations / Study areas	Density	Frequency (%)	Mean production of a base (g)	Mean of the largest canopy cover (cm)	Mean of the smallest canopy cover (cm)	Mean of plant height (cm)
Damgahan	0.17	10.00	31.2	25.4	22.2	24.6
Deh Bala	0.37	23.33	38.09	39.2	33.4	43
Taghi Abad	0.27	16.67	29.75	32.4	27.2	32.57
Tezerjan	0.2	10.00	30.2	24.9	17.7	26.4
Zardein	0.2	13.33	23.83	31.5	25.2	33.1

of plants in desert areas. The soil characteristics play a key role in plant metabolites. In this regards EC and pH are more significant than others (Danin, 2008). The present result approved these points completely. The significant variety in sand, silt, and clay caused different soil texture. Soil textures of Taghi Abad, Deh Bala, Damgahan, Zardein, and Tezerjan were Silt-Loam, Silt-Loam, Sandy-Loam, Sandy-Loam, and Loamy-Sand, respectively. There are some differences between current results with previous (Azimi, 2009).

Vegetation Variation

According to transects and plots in the study areas, the calculated factors showed some difference in various habitat. As can be seen in Table 4, Deh Bala showed the highest level in tested parameters (density, frequency, and production). It could be said that these results obtained in Deh Bala and Taghi Abad where had maximum rainfall, lowest temperature and one of the best habitats in soil nutrient elements in contrast with others. Different phenological stages are affected by biotic and abiotic factors. For this purpose, the genus was investigated in different habitats and each habitat showed various result (Table 5). In following, co-dominant plants were registered. Table 6 shows co-dominant plants in each area.

Habitat 1: Taghi Abad, 2: Damgahan, 3: Deh Bala,
4: Zardein 5: Tezerjan

Physiological Characteristics

Chlorophylls and carotenoids are critical components of the photosynthetic machinery. Their role in harvesting light energy, stabilization of membranes, and energy transduction has been studied extensively (García-Valenzuela et al., 2005; Ciganda et al., 2008; Porcar-Castell et al., 2014). In order to revealed simple and interaction effect of phenological stages and studied areas, Analysis of Variance was done (Table 7). As can be seen in Table 8 and 9, the phenological stages and different geographical areas were significant on the studied pigment factors except chlorophyll b. In other words, vegetative stage and variation in habitat had no effect on chlorophyll b while the other studied pigment had significant deviation. Because of some significance, the mean comparisons of studied areas and vegetative stages have been done. Tables 8 and 9 show the mean comparisons. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were varied between 9.73 – 13.18, 2.02 – 2.88, 11.76 – 15.98, and 4.48 – 5.95 mg/g, respectively. Based on statistical analysis, the best habitats for the studied pigments were Deh Bala and Taghi Abad. In all population, the pigments in the vegetative stage were lower than that in full flowering period. The present results have some difference with the study on *Sorghum bicolor* L. (Azari Nasrabad et al., 2017). They revealed that the impact of drought in the phenological and reproductive growth stages was different and the contents of chlorophyll and carotenoid were reduced. In following, the different rainfall in various studied areas showed interesting results. The available moisture

Table 5. Phenology of *Z. clinopodioides* in different habitats.

	Phenology	Vegetative					The appearance of flower spikes					Full flowering					Seed processing				
	Habitat Period	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
March	1-10				*																
	11-20		*		*																
	21-31	*	*	*	*																
April	1-10	*	*	*	*	*															
	11-20	*	*	*	*	*		*		*											
	21-30			*		*	*	*	*	*	*										
May	1-10						*	*	*	*	*		*		*						
	11-20						*		*		*	*	*	*	*						
	21-31											*	*	*	*	*					
June	1-10											*		*		*	*	*		*	
	11-20																*	*	*	*	
	21-30																*	*	*	*	*
July	1-10																	*		*	*

	Phenology	Full seedling					Seed loss					Leave loss					Dormancy				
	Habitat Period	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
June	21-30	*		*																	
July	1-10	*	*	*	*																
	11-20	*	*	*	*																
	21-31		*		*	*	*	*	*	*											
August	1-10					*	*	*	*	*											
	11-20					*	*	*	*	*											
	21-31					*	*	*	*	*	*	*		*	*						
September	1-10										*	*	*	*	*						
	11-20										*	*	*	*	*	*				*	
	21-30															*	*	*	*	*	*
October																	*	*	*	*	*
–																	*	*	*	*	*
February																	*	*	*	*	*

Table 6. Co-dominant plants.

Plant name	Family	Life form	Area
Acantholimon incomptum Bioss. &Buhse.	Plumbaginaceae	Ch	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Acantholimon nigricans Mobayen	Plumbaginaceae	Ch	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Acanthophyllum laxiusculum Schiman-Czeika	Caryophylaceae	Ch	Taghi Abad, Zardein
Achillea wilhelmsii C. Koch	Asteraceae	He	Taghi Abad, Deh Bala, Zardein, Damgahan
Amygdalus lycioides Spach.	Rosaceae	Ph	Zardein
Artemisia aucheri Boiss.	Asteraceae	Ch	Taghi Abad, Deh Bala, Damgahan
Artemisia persica Boiss.	Asteraceae	Ch	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Artemisia sieberi Besser.	Asteraceae	Ch	Thaghi Abad, Deh Bala
Astragalus spachianus Boiss. &Buhse.	Papilionaceae	He	Taghi Abad, Damgahan
Berberis integerrima Bunge.	Berberidaceae	Ph	Damgahan
Carex physodes M. B.	Cyperacea	Cr	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Clematis ispahanica Boiss.	Ranunculaceae	Ph	Damgahan
Cotoneaster persica Pojark.	Rosaceae	Ph	Zardein
Cousinia onopordioides Ledeb.	Asteraceae	He	Taghi Abad, Zardein, Tezerjan
Echinops aucheri Boiss.	Asteraceae	He	Zardein, Tezerjan
Eryngium bungei Boiss.	Apiaceae	He	Zardein
Glycyrrhiza glabra L.	Papilionaceae	He	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Helichrysum davisianum Rech. f.	Asteraceae	He	Deh Bala, Damgahan
Hyoscyamus pusillus L.	Solanaceae	Th	Tezerjan
Juncus bufonius L.	Juncaceae	Cr	Taghi Abad
Marrubium vulgare L.	Labiateae	He	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Melica jacquemontii Decne. ex Jacquem	Gramineae	Cr	Taghi Abad
Melica persica Kunth.	Gramineae	Cr	Taghi Abad
Nepeta persica Bioss.	Labiateae	He	Zardein, Tezerjan
Phagnalon nitidum Fres.	Asteraceae	He	Damgahan
Pimpinella aurea DC.	Apiaceae	He	Deh Bala, Zardein, Tezerjan
Plantago ciliate Desf.	Plantaginaceae	He	Deh Bala
Plantago lanceolate L.	Plantaginaceae	He	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Rumex dentatus L.	Poligonaceae	Th	Zardein
Salvia macrosiphon Boiss.	Labiateae	He	Damgahan
Sanguisorba minor Scop.	Rubiaceae	He	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Scutellariam ulticaulis Boiss.	Labiateae	He	Tezerjan, Deh Bala, Taghi Abad
Silene gynodioica Ghazanfar	Caryophyllaceae	Th	Zardein, Damgahan, Taghi Abad, Deh Bala, Tezerjan
Stachys inflate Benth.	Labiateae	He	Tezerjan, Deh Bala, Zardein
Stachys obtusicrena Boiss.	Labiateae	He	Tezerjan, Deh Bala, Zardein
Stipa barbata Desf.	Poaceae	He	Tezerjan, Deh Bala, Zardein
Teucrium polium L.	Labiateae	He	Damgahan, Deh Bala
Trifolium alexandrinum L.	Papilionaceae	He	Zardein
Trifolum pretense L.	Papilionaceae	He	Deh Bala, Zardein
Verbascum songaricum Schrenk ex Fisch. &C. A. Mey.	Scrophulariaceae	He	Deh Bala, Tezerjan
Ziziphora clinopodioides Lam.	Labiateae	He	Taghi Abad, Deh Bala

Table 7. ANOVA for dissimilar phenological stage and areas.

SOV	Df	chl a	chl b	chl ab	Ca	Pro	SS
Pheno	1	29.66 *	0.604 n.s	38.71 **	2.80 *	0.00096 **	0.020 **
Stu areas	4	11.42 *	0.686 n.s	17.55 *	2.03 *	0.00011 n.s	0.002 *
Pheno × Stu areas	4	1.18 n.s	0.028 n.s	1.40 n.s	0.25 n.s	0.00011 n.s	0.014 **
EE	20	4.29	0.309	5.93	0.816	0.00008	0.00096

n.s: not significant, *: $P \leq 0.05$, **: $P \leq 0.01$, SOV: Source of the variation, chl a: chlorophyll a, chl b: chlorophyll b, chl ab: chlorophyll a + chlorophyll b, Ca: carotenoid, Pro: prolin, SS: soluble sugar, Pheno: phenology, Stu areas: studied areas, EE: experimental error.

Table 8. Mean comparisons of different phenology.

Traits treatments	chl a	chl b	chl ab	Ca	Pro	SS
Vegetative	10.97 b	2.46 a	13.44 b	5.12 b	0.360 b	0.182 b
Flowering	12.96 a	2.74 a	15.71 a	5.73 a	0.371 a	0.234 a

Table 9. Mean comparisons of studied areas.

Study areas	chl a	chl b	chl ab	Ca	Pro	SS
Damgahan	11.78 ab	2.67 ab	14.46 ab	5.42 ab	0.36 a	0.18 b
Deh Bala	13.18 a	2.80 a	15.98 a	5.86 a	0.37 a	0.23 a
Taghi Abad	13.02 a	2.88 a	15.90 a	5.95 a	0.36 a	0.22 a
Tezerjan	12.14 ab	2.64 ab	14.78 a	5.40 ab	0.37 a	0.18 b
Zardein	9.73 b	2.02 b	11.76 b	4.48 b	0.36 a	0.20 ab

had direct relationship with the studied pigments. Other researchers confirmed this relationship (Fifaei et al., 2016; Azari Nasrabad et al., 2017). Furthermore, phenological stages × studied areas revealed no significant effect on the pigments in the present study.

Accumulation of different active ions, sugars and amino acids like proline is responsible for osmotic adjustment in plant cells (Konigshofer & Loppert, 2015). Osmotic adjustments maintain turgor pressure, control cell expansion, and growth as well as stomatal aperture, photosynthesis, and water flow during water shortage periods (Ruiz-Lozano, 2003). Based on the key role of proline, it had been decided to investigate it in the diverse situation. Proline content was significantly affected by phenology period while different regions did not affect it in the present study. Moreover, the interaction between phenology and studied areas had no significantly effect on proline content. The means of proline in the vegetative and flowering periods were 0.360 and 0.371, respectively. The mean of proline in the dissimilar area had very low variation (0.36 – 0.37). Sharifi Yazdi et al. (2013) discovered the same results, too (Sharifi Yazdi et al., 2013). They believed that the amount of proline increased with each step. In other words, the amount of proline has a direct relationship with phenology. Gideon et al. (2016) studied the proline content of three pepper species in response to water stress imposed at different stages of growth. Their results showed that the concentration of proline in leaves of three pepper species was found to be remarkable at different stages of growth. These results were the same as the present results. It should be said that plants have the ability to accumulate non-toxic compounds such as proline that protects cell damage due to the drought stress or low water potential of cells. Also, the osmotic pressure of the plant cell regulates many processes through the accumulation of non-toxic solutes inside the cell (Umezawa et al., 2006; Lipiec et al., 2013).

Results of the present study indicated that the only factor, which was affected by phenology, different area, and interaction of phenology and different area, was soluble sugar content. In addition, the obtained results showed that the lowest amount of soluble sugar was observed in Tezerjan and Damgahan (0.18) while the highest one was for Deh Bala and Taghi Abad (0.22 and 0.23). It should be said that the incremental trend of soluble sugar had a direct relationship with a phenology. In other words, the quantity of soluble sugar at the flowering stage was more than that in the vegetative stage. These results were in accordance with the investigation on *Halocnemum strobilaceum* and *Halostachys caspica* (Arzani et al., 2007; Sharifi-Rad et al., 2017).

Conclusions

In order to find the best growth situation, it is reasonable to investigate different species in their natural habitats. Investigation on *Ziziphora clinopodioides* Lam. subsp. *rigida* (Bioss.) Rech. f. in different habitats showed the concerted impact of changes in temperature, precipitation and topography on photosynthetic pigments and some physiological factors. For example, phenological stages and climatic conditions significantly affected on the studied pigments except chlorophyll b. The interaction between phenological stages and environmental conditions effected on soluble sugar significantly. In following, physiological factors affected growth strategy. One of the most important environmental factor is available water, which has a great degree of control of growth responses and photosynthesis level. On the other hand, the influence of precipitation patterns and amounts in the long term will be of importance for the physiological factors. Briefly, ecological factors and their impacts depend on the variation in climate thus their strength may affect the year to year responses to a different degree. Based on the present result, it is revealed that the best habitats for the studied species were Deh Bala and Taghi Abad. According to the ecological characteristics in Taghi Abad and Deh Bala, it could be suggested two future investigations. The first one is the effect of bio fertilizer in different phenological stage and the other one is plant water requirement.

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