ISSN: 2538-2152; ECOPERSIA 2023;11(2):93-104.

Effects of Phenological Stages and Ecological Factors on Secondary Metabolites of *Clematis ispahanica* Boiss

[DOR: 20.1001.1.23222700.2023.11.2.6.8]

[DOI: 10.22034/ecopersia.11.2.93]

ARTICLEINFO

Article Type
Original Research

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How to cite this article
Ashrafzadeh M., Niknahad – Gharmakher H. Saharkhiz MJ., Ghorbani Nohoji M. Effects of Phenological stages and Ecological factors on secondary metabolites of *Clematis ispahanica* Boiss. ECOPERSIA 2023;11(2): 93-104
DOR:
20.1001.1.23222700.2023.11.2.6.8

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Article History
Received: May 3, 2023
Accepted: June 6, 2023
Published: February 15, 2023

ABSTRACT

Aims: This research aimed to evaluate some phytochemical characteristics of *Clematis ispahanica* Boiss. in two arid and semi-arid sites.

Materials & Methods: After studying the climatic conditions in each site, 30plots ($6m^2$) were established randomly– systematically for soil and plant sampling. A total of ten composite soil samples were collected from two depths (0–10 and 10-30 cm), and some of their properties were measured. Three plant samples were obtained and mixed at three Phenological stages in each site, and their secondary metabolites were determined. A comparison of soil properties between the two sites was performed using an independent-sample t-test. Phytochemical comparison of plants between two sites and three Phenological stages was performed using Factorial analysis of variance and Duncan's multiple range test. Correlation between soil properties and phytochemical characteristics was performed using the Pearson correlation coefficient.

Findings: The results demonstrated that *C. ispahanica* has higher secondary metabolites (total Phenol, total Flavonoid, and total Alkaloids) in semi–arid climates compared to arid climates. In both sites, the highest content of secondary metabolites was observed at the flowering stage (Phenolic content in Bavanat and Mehriz city's 184.33 & 115.16 mg GAE.g DW Ext, Flavonoid content, 32.57 & 28.37 mg QE.g DW Ext, Alkaloids content 12.89 & 5.75 mg Atr.g DW Ext, respectively). A significant, mostly positive, correlation was observed between the phytochemical characteristics of *C. ispahanica* and soil pH, EC, and total Nitrogen.

Conclusion: It was concluded that heavier soil texture, semi-arid climate, and flowering stage are more suitable for *C. ispahanica* secondary metabolites production.

Keywords: *Clematis ispahanica*; Climate; Phytochemical Characteristics; Soil Properties.

CITATION LINKS

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[DOR: 20.1001.1.23222700.2023.11.2.6.8]

[DOI: 10.22034/ecopersia.11.2.93]

Introduction

In addition to essential primary metabolites (Carbohydrates, Lipids, and Amino Acids), plants produce large amounts of low molecular weight components called secondary metabolites (SMs). SMs do not play a significant role in the early processes of plant life, but they are essential in their adaptation to the surrounding environment [1].

SMs have antibacterial, antifungal, and antiviral properties protecting the plant from pathogens [2]. These compounds have an important role in absorbing ultraviolet light and protecting leaves against the harmful effects of this radiation. Moreover, SMs can prevent plants from herbivores and other competing plants and sometimes lead to fertility disorders in consumer animals [3]. SMs use as communication signals between some plants and symbiotic microorganisms, and some plants serve them to attract seed dispersers and pollination [4]. The production of SMs is often less than 1% of the plant's dry weight and depends significantly on the plant's Phenological and physiological stages [2]. Although genes control the amount of SMs, their concentration and accumulation are influenced mainly by environmental conditions [4].

The quantity, quality, and composition of SMs are influenced by the harvest season, the climate [5], geographic features [6], harvest time [7], extraction method [8], plant Phenological stage [9], soil properties, plant organs, age and growth stages. These factors influence plant growth [10], physiology, and morphology [11] and cause changes in plant genetic ecology [12].

It has been stated that the advantageous medicinal effects of particular plants usually result in the combinations of their SMs [13]. Antioxidants usually inhibit the synthesis of free radicals or reduce their activity. Antioxidants not only confront active oxygen types but also minimize their sourness, reduce the formation of toxic substances from oxidation products, maintain food quality, and extend their shelf life. Antioxidants prevent many diseases, such as cancer and cardiovascular disease [14]. Free radicals are the most critical factors in food oxidation, which, with a destructive process, cause the loss of their nutritional value and change in their chemical composition. Sources of antioxidants in plants are Phenols [15]. Phenolic compounds such as Flavonoids, Terpenoids, and Tocopherols have strong Antioxidant activity [16]. Flavonoids are a group of Phenolic compounds that can inhibit free radicals and oxidative enzyme activity. Moreover, they have analgesic and anti-inflammatory properties. Flavonols and their derivatives (Flavonoids) are found freely or in combination with glycosides in many plants. Also, Alkaloids are naturally Nitrogen-containing active organic compounds of medicinal plants with widespread pharmacological activities. It has been demonstrated that more than 140 Angiosperm plant families and 20,000 genera are rich in Alkaloids [17].

Regarding the impact of the climate on SMs, limited researches are available due to the wide- range and complexity of this subject [18, 19]. Climate affects the amount of SMs by affecting the amount of CO_2, radiation, temperature, and rainfall [18]. Oloumi and Hassibi (2011) stated that climatic conditions affect the changes in SMs of *Glycyrrhiza glabra*. Some studies have revealed that plants grown in dry climates have more SMs than those grown in temperate climates [21]. The investigation on the effect of soil texture on the amount of some compounds related to the antioxidant activity of *Cynara scolymus* demonstrated that soil properties affect the qualitative characteristics of *Cynara scolymus* leaf [22].

Clematis ispahanica Boiss. is an endemic perennial species of Iran belonging to the buttercup family (Runanculaceae) used in herbal

medicines for health effects. Various species of this genus are known as valuable traditional medicinal plants because of different medicinal constituents, including glycoside, saponins, and Alkaloids [23]. Recently some studies were defined on evaluating antioxidant activity along with the total Phenolic and Flavonoid content of *C. ispahanica* in Iran. As a result, it is demonstrated that the plant is a rich source of antioxidant compounds, so that it can be regarded as a good choice for medical and industrial uses [24]. Evaluation of the content and performance of some active ingredients extracts of *Citrullus colocynthis* organs demonstrated that habitat, Phenological stage, and organ of the plant have a significant effect on antioxidant activity, Phenol content, and total Flavonoid of this plant, separately ($p<0.01$). The results revealed that interaction between habitat × plant's organ and habitat × Phenological stages significantly affects all Phenolic and antioxidant compounds ($p<0.05$). The results indicated that the plant extract has the highest Phenolic and Flavonoid compounds and antioxidant activity in the flowering stage [13]. Considering the effect of different sites and climates on the number of SMs, as well as the need to identify the medicinal value of plant species, the aim of present study was aimed to investigate the effects of climate, site, and different Phenological stages on the number of SMs (total Phenol, total Flavonoid, total Alkaloids) and some phytochemical properties (antioxidant activity) of *C. ispahanica*.

Materials & Methods

Study Area

This study was conducted in two sites (Mehriz in Yazd Province and Bavanat in Fars Province) in Central Iran in 2017 (Figure 1).

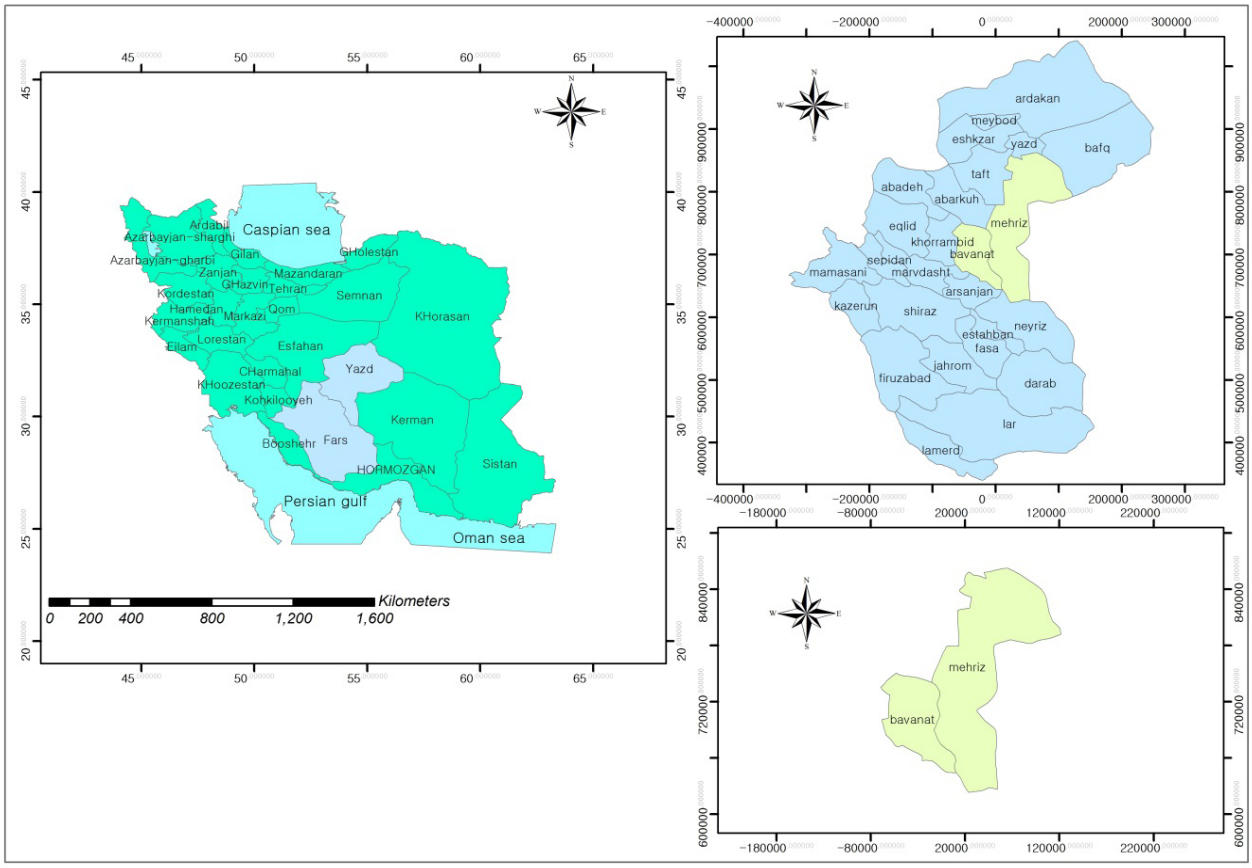

Figure 1) Location of the study sites in the south of Iran.

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[DOI: 10.22034/ecopersia.11.2.93]

In the studied sites, the rainy season is generally from November to May, and the dry season is from May to October (Table 1).

Soil and Plant Sampling Method

In the key areas of each site, 30 plots (6m^2) were established randomly– systematically for soil and plant sampling. Two soil samples were collected at a depth of 0–10 and 10-30 cm from each plot. One composite soil sample was obtained from each three soil samples. A total of ten composite soil samples were collected from each depth of the studied sites. In the laboratory, soil texture was determined using the hydrometer method. Soil pH and electrical conductivity (EC) were determined by a pH meter and conductivity meter, respectively. The Walkley- Black and Kjeldahl methods determined soil organic Carbon (OC) and total Nitrogen (N). Exchangeable Potassium (K) was determined using a flame Photometer. Exchangeable Calcium and soil lime percentage were obtained by titration method. Adsorbable Phosphorus was measured using the Olsen method. The details of mentioned methods were described elsewhere [25]. To study the phytochemical properties of *C. ispahanica*, the Leaves of this plant were clipped at three

Table 1) Normal environmental characteristics of the studied sites.

Environment Factors	Site	
	Mehriz	Bavanat
Location	54° 18’ 15 "E, 31° 28’ 52” N	53° 45’ 43 “E, 30° 12’ 14” N
Altitude (m)	1880-3873	2240-3215
Mean Annual Rainfall (mm)	59	270
Mean Annual Temperature (°C)	18.5	17
Climate	Arid	Semi-arid
Depth (Cm)	0-10, 10-30	0-10, 10-30
Soil Texture	Sandy clay loam and Sandy loam	Clay loam and Loam

Table 2) Three Phenological stages and climate indicators in two studied sites.

Site	Phenological Stage	Climate Indicators			Month
		Temperature (^{0}C)	Moisture (%)	Precipitation (mm)	
Bavanat	Growth	18	57.2	10.4	Early May
	Flowering	23	44.2	0	August
	Maturity	20.5	32	0	September
Mehriz	Growth	20	35.2	6	Early April
	Flowering	28	28	0	Early June
	Maturity	29.3	20.6	0	August

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Phenological stages on 15th April (vegetative growth), 15th June (flowering), and 15th August (maturity) in mentioned plots. All samples were dried in the dark at room temperature and powdered for phytochemical analysis. One composite plant sample was obtained from every ten plots, and its total antioxidants, total Phenol content, total Flavonoid, and total Alkaloids were determined.

Phytochemical Analysis

Preparation of Plant Extracts

Five grams of the powdered dry plant was mixed with 50 ml Methanol and extracted using an Ultrasonic instrument for 30 minutes. After filtration of the extract using filter paper, it was condensed using rotary and vacuum at 40˚C until obtaining dry extract [22].

Measurement of Total Phenolic Content

The amount of total Phenolic content was evaluated using the Folin-Ciocalteu reagent [26]. Gallic acid was used as a standard for determining total Phenolic content. It was expressed as mg.g-1 Gallic Acid Equivalent (GAE). Concentrations of 5, 10, 30, 50, 100, 250, 500, 1000, 5000, and 10000 mg/ml-1 of Gallic acid were prepared in Methanol. A 1mg/ml concentration of plant extract was prepared in Methanol, and 20µl of the sample was placed into the test. It was mixed with 100µl of Folin-Ciocalteu reagent. The mixture was added to 300ml of 7.5% Sodium Carbonate. The tubes were shaken with a shaker for 2 hours at room temperature before taking the absorbance with a Nano-Drop Spectrophotometer, EPOCH Model, Bio Tek Co. in 760 nm. Reading was done in five replicate. The total Phenol content was represented as mg GAE/Li of extract.

Measurement of Total Flavonoid Content

The Aluminum Chloride method measured total Flavonoid content [27]. At first, the extract of *C. ispahanica* was prepared at a concentration of 500 mg / 1000 ml of Methanol. Then, 600 µl of Aluminum Chloride 2% was added to 600 µl of the extract, and the samples were prepared in three replicates. After 10 minutes, the absorbance of the samples was measured at 420 nm with a NanoDrop Spectrophotometer. Total Flavonoid contents were calculated using a standard Quercetin curve prepared at concentrations of 5, 10, 50, 100, 250, 500, and 1000 mg per 1000 ml and reported as mg Quercetin per gram of dry extract.

Evaluation of Antioxidant Activity

The antioxidant activity of *C. ispahanica* was determined according to 2-Diphenyl- Picryl-Hydrazy (DPPH) method [26]. At first, Methanolic extract was prepared with different concentrations of 10000, 3000, 1000, and 300 mg.ml-1 in Methanol solvent. DPPH was also prepared at a concentration of 400 mg.ml-1 Methanol.

200 µl of DPPH and Methanol in a ratio of 1 to 1 and 40 µl of mentioned concentrations of Methanolic extract were mixed, and each one was added to special plates in three repliCations.

Samples were placed in the dark and shaken for one hour. The absorbance of the specimens was measured at 517 nm with a Nano-Drop Spectrophotometer. The samples' reception scavenging capacity of free radical (RSC) was obtained using the following equation.

$$RSC(\%) = 100 \times \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \quad \text{Eq. (1)}$$

where, A_{blank} and A_{sample} are the absorbance of the control sample and tested sample, respectively. The activity of the extracts was compared using the IC50 parameter, a concentration of extract that inhibits 50% of free radicals, too [26].

Measurement of Total Alkaloid Content

Initially, one gram of the powdered sample was placed into a 250 cc Erlenmeyer flask,

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and five cc ammonia 25%, 25 cc Methanol, and 75 cc chloroform were added, and its lid was closed firmly. The Erlenmeyer flask was placed in the ultrasonic apparatus for 10 minutes and then transferred to a dark place for half an hour. Finally, the solution was filtered using filter paper. The filtered solution was poured into a 200 cc balloon to remove solvents and connected to a rotary machine. To dissolve all of the remaining sediments, 25 cc chloroform and ten cc sulfuric acid 1N were added, and ultrasonic equipment was used for a short time. The solution was transferred to a decanter, and after creating two distinct phases, the chloroform phase (lower phase) was removed from the decanter, and the aqueous phase (upper phase) remained. Then, the acidity (pH) of the solution was adjusted to 10-11, using ammonia 25%. Extraction was performed using chloroform 25 cc. The chloroform phase (lower phase) was collected in smaller balloons, and the aqueous phase (upper phase) was re-extracted and collected using 10cc chloroform. Na_2SO_4 was used to dehydrate this collected extract and filter. Finally, the solvent (chloroform) was removed using a rotary machine. 1mg of the sample extract was dissolved in 1 ml HCL 2N and filtered to measure total Alkaloids. The pH of the extract was neutralized using Sodium hydroxide 0.1 N. One ml of this solution was transferred to a decanter. Five ml of bromocresol green and 5 ml of phosphate buffer were added and shaken. The formed mixture was extracted further using twice 5ml chloroform. The volume of extracted chloroform phase reached 10 ml using chloroform. The absorbance of the complex in chloroform was measured at 415 nm using a NanoDrop Spectrophotometer [28].

Preparation of Standard Curve

The standard curve of Atropine (1 mg /10 ml) with concentrations of 500, 1000, 1500, 2000, 2500, 3000, and 5000 ppm was prepared, and a calculation of total Alkaloids were reported per mg of Atropine in one gram of dry extract [29].

Statistical Analysis

A comparison of soil properties between the two sites was performed using an independent sample t-test. The correlation between soil properties and phytochemical characteristics of *C. ispahanica* was performed using Pearson's correlation coefficient. Phytochemical analysis (between two sites and three Phenological stages) was performed using Factorial analysis of variance, and Duncan's multiple range test did mean comparisons. All analyses were done using $SPSS_{18}$ software.

Findings

Effects of Sites, Phenological Stage, and Their Interaction on SMs

According to the results, sites, Phenological stage, and the interaction between sites and Phenological stages have a significant effect ($p<0.05$) on antioxidant activity and total Alkaloids of *C. ispahanica*. (Table 3). The results demonstrated a significant effect ($p<0.05$) of sites and Phenological stage on the total Phenolic content of *C. ispahanica*, while only sites have a significant effect ($p<0.05$) on the total Flavonoid content of this species. No significant interaction ($p>0.05$) between sites and Phenological stage has been observed in terms of total Phenolic and Flavonoid content (Table 3).

Means Comparisons of SMs

According to the results (Table 4), the highest (551.46 % g. DW Ext^{-1}) and lowest (182.71 % g. DW Ext^{-1}) amounts of antioxidant activity were observed at plants at flowering and maturity stages in (semi-arid) sites. Moreover, the highest amount of total Alkaloids (12.89 mg $Atr.ml^{-1}$) was observed at the flowering stage in the Bavanat site, and the lowest (1.82 mg $Atr.ml^{-1}$) was observed at

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Table 3) The result of the Factorial analysis of variance between site and Phenological stage, as well as their interactions on the SMs of *C. ispahanica*.

Secondary Metabolites	Source	Df	F	Sig.
Total Phenol (mg GAE.g DW Ext)	Site	1	11.083	0.00
	Phenological stage	2	5.343	0.01
	Phenological stage× Site	2	3.439	0.054
Total Flavonoid (mg QE.g DW Ext)	Site	1	5.863	0.02
	Phenological stage	2	0.008	0.99
	Phenological stage× Site	2	1.078	0.36
Antioxidant Activity (g. DW Ext%)	Site	1	0.734	0.04
	Phenological stage	2	21.775	0.00
	Phenological stage× Site	2	13.679	0.00
Total Alkaloid (mg Atr.g DW Ext)	Site	1	19.00	0.00
	Phenological stage	2	102.16	0.00
	Phenological stage× Site	2	38.55	0.00

the maturity stage.

The results revealed that the Phenol content of *C. ispahanica* only in the Bavanat site changed significantly in different Phenological stages, so the highest Phenol content (184 mg GAE.g^{-1} DW Ext)) was observed at the flowering stage, and the lowest (102.83 mg GAE.g^{-1} DW Ext) was observed at the maturity stage. No significant change in Phenolic content was observed in different Phenological stages of *C. ispahanica* in Mehriz (arid) site. Regarding total Flavonoid content, only one site had a significant effect. *C. ispahanica* in the Bavanat site had higher total Flavonoids in all Phenological stages than the Mehriz site (Table 4).

Comparisons of Soil Properties in Two Studied Sites

A significant difference was observed between the soil pH and $CaCo_{3\ mean,}$ so their means in the Mehriz site were significantly higher than in the Bavanat site (Table 5). Our results revealed that the mean of adsorbable Phosphorus and organic Carbon in the first depth (0-10cm) and second depth (10-30cm) of the Bavanat site were significantly ($P<0.05$) higher than the Mehriz site, respectively. No significant differences were observed between other soil physical properties (Table 5).

Correlation between Soil Properties and Secondary Metabolites of *C. ispahanica*

In the Bavanat site, a significant negative correlation coefficient between total Phenolic

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Table 4) Analysis of variance of 4 phytochemical properties at three Phenological stages in two sites.

Site	Phenological Stage	Secondary Metabolites			
		Phenolic Content (mg GAE.g DW Ext)	Flavonoid Content (mg QE.g DW Ext)	Antioxidant Activity (g. DW Ext%)	Alkaloid (mg Atr.g DW Ext)
Bavanat	Growth	14819.79± ^{ab}	31.0793±^{a}.2	75.25630.14±^{bc}	3.320.93±^{cd}
	Flowering	184.3316.04±^{a}	32. 570.75± ^{a}	551.4661.77± ^{a}	12. 891.25±^{a}
	Maturity	102.8311.85±^{b}	29.61.73± ^{a}	182.719.34±^{c}	1.820.24±^{d}
Mehriz	Growth	106.833.99±^{a}	26.973.49±^{b}	41. 32386.23±^{b}	4.231.36±^{bc}
	Flowering	115.1618.77±^{a}	28.371.90±^{b}	348.2534.62±^{b}	5.711.75±^{b}
	Maturity	98.55.3±^{a}	24.950.83±^{b}	248.0817.98±^{bc}	2.491.54±^{d}

Lowercase letters show comparisons of columns (between Phenological stages in studied sites)
GAE: Gallic Acid Equivalent, DW: Dry extract, QE: Quercetin, Atr: Atropine

and total Flavonoid content with pH of soil first depth (0-10 cm) was observed, While there was a significant negative correlation coefficient between EC of soil first depth (0-10 cm) and total Alkaloids of *C. ispahanica*. It is worth mentioning that a significant positive correlation coefficient between the Phenolic content of *C. ispahanica* and total soil Nitrogen was also observed (Table 5).
Between soil properties of second depth (10-20cm) in the Bavanat site, there was a significant positive correlation coefficient between soil total Nitrogen and total Alkaloids of *C. ispahanica*. In the Mehriz site, a significant positive correlation coefficient between total Alkaloids and exchangeable soil Calcium in the first depth and a significant positive correlation coefficient between Phenol content and soil Ec in the second depth were observed (Table 6).

Discussion

The growth stage and climatic characteristics are influential factors in the production of SMs plants [30]. So, in addition to environmental stresses, the increase of secondary metabolites of *C. ispahanica* can be due to its important physiological and structural changes during the development stages [23]. In the present study, the highest SM levels of SMs were observed at the flowering stage, coinciding with the onset of environmental stresses (dryness and high temperature) in the studied sites. This could be due to plant response to drought stress [31]. Environmental stresses affect the quantity and quality of plant SMs [32]. For most plants, environmental factors such as temperature, soil fertility, and soil water can significantly affect their ability to produce SMs, finally altering total phytochemical profiles [33]. An increase in plant SMs has been reported due to drought [33]. When the plant is under stressful conditions caused by environmental stress, the production of secondary metabolites may increase. An increase in secondary metabolites is often associated with increased photosynthesis, in which the produced Carbon is allocated to secondary compounds such as Phenol and glycosides instead of being used

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Table 5) Means comparison of soil parameters in two studied sites.

Depth (cm)	Soil Properties	Site		Source	
		Bavanat	Mehriz	Sig.	t
0-10	pH	8.520.24±	0.25±8.85	0.01*	-2.89
	Ec (dSm^{-1})	0.160.04±	0.220.15±	0.26 ns	-1.14
	Exchangeable Calcium (ppm)	3.630.88±	4.682.66±	0.15 ns	-1.48
	Exchangeable Magnesium(ppm)	6.11.08±	2.441.72±	0.20 ns	-1.30
	Exchangeable Potassium (ppm)	1.991.09±	2.391.12±	0.43 ns	-0.80
	Exchangeable Sodium (ppm)	1.820.51±	1.730.75±	0.75 ns	0.31
	Organic Carbon (%)	0.830.22±	0.730.43±	0.51 ns	0.66
	$CaCo_3$ (%)	43.055.71±	58.1513.85±	0.00*	-3.18
	Adsorbable Phosphorus (ppm)	26.8122.32±	15.725.51±	0.01*	-2.90
	Total Nitrogen (%)	0.150.05±	0.110.07±	0.21 ns	1.29
	Sand (%)	69.201.75±	75.101.54±	0.77 ns	-2.52
	Silt (%)	19.951.42±	17,751.58±	0.38 ns	1.03
	Clay (%)	10.85± 0.70	7.140.47±	0.32 ns	4.34
10-30	pH	8.730.10±	9.060.18±	0.00*	-4.94
	Ec (dSm^{-1})	0.150.05±	0.160.07±	0.78 ns	-0.27
	Exchangeable Calcium (ppm)	3.642.02±	4.122.19±	0.61 ns	-0.50
	Exchangeable Magnesium(ppm)	2.881.54±	3.641.67±	0.30 ns	-1.05
	Exchangeable Potassium (ppm)	2.911.64±	1.970.85±	0.12 ns	1.60
	Exchangeable Sodium (ppm)	1.360.30±	1.170.43±	0.27 ns	1.13
	Organic Carbon (%)	0.820.36±	0.340.23±	0.00*	3.52
	$CaCo_3$ (%)	42.909.29±	62.2513.66±	0.00*	-3.62
	Adsorbable Phosphorus (ppm)	22.0915.31±	18.0314.62±	0.55 ns	0.60
	Total Nitrogen (%)	0.120.06±	0.100.12±	0.63 ns	0.48
	Sand (%)	67.801.29±	77.401.53±	0.32 ns	-4.77
	Silt (%)	23.151.34±	15.151.29±	0.61 ns	4.28
	Clay (%)	9.05± 0.66	7.45± 0.42	0.19 ns	2.02

*P < 0.05, ns, not significant.

Table 6) Pearson correlation coefficients (r) for Secondary metabolites with soil properties.

Secondary Metabolites	Bavanat				Mehriz		
	Depth(cm) 0-10		Depth (cm) 10-30		Depth (cm) 0-10	Depth (cm) 10-30	
	EC	pH	N	N	--	Ca	EC
Total Phenols	0.15 ns	0.71*	0.73*	0.22 ns	--	0.35 ns	0.64*
Total Flavonoids	0.24 ns	-0.76*	0.42	0.19 ns	--	-0.15 ns	0.27 ns
Antioxidant activity	-0.07 ns	0.18 ns	-0.52	0.35 ns	--	-0.04 ns	-0.58 ns
Total Alkaloids	-0.36*	0.33 ns	-0.56 ns	0.76 ns	--	0.86*	-0.47 ns

* = P < 0.05 and ns = no significant

[DOR: 20.1001.1.23222700.2023.11.2.6.8]
[DOI: 10.22034/ecopersia.11.2.93]

[DOR: 20.1001.1.23222700.2023.11.2.6.8]

[DOI: 10.22034/ecopersia.11.2.93]

in plant growth [19]. Plants have different physiological and morphological responses to physical and chemical factors. Increasing the Nitrogen content compounds of the plant (antioxidants, Phenolic compounds, Flavonoids, and Alkaloids) is a physiological defense mechanism of the plant against environmental stresses [2]. Reactive Oxygen types are produced during environmental stresses, which can cause chlorophyll degradation and reduce photosynthesis [34]. Increasing the production of Phenolic compounds prevents the free radicals penetration into the cell and limits the peroxidation reaction [35]. In most plants, antioxidant activity is directly related to Phenolic and Flavonoid compounds [36].

Changes in moisture content cause changes in the rate of soil microbial activity, which causes a change in the intensity of organic matter decomposition [37]. It can be argued that the soil of the Bavanat site (semi-arid climate) has higher humidity due to its heavier soil texture and higher precipitation than the Mehriz site (arid climate), so it has higher microbial activity and organic Carbon percentage (Table 5). Significant higher soil pH and $CaCo_3$ were observed in the Mehriz site (arid climate). In arid climates, the accumulation of soil alkaline Cations is higher than in semi-arid climates, causing an increase in soil pH [25]. Differences in terms of companion plants of *C. ispahanica* in the studied sites have led to a difference in the quality of the fallen litter to the soil and, consequently, the difference in soil microorganisms that can lead to changes in soil pH. It has been reported that some plants such as *Haloxylon sp., Anabasis sp.,* and *Seidlitzia sp.* realize some alkaline Carbonate and bi-Carbonate due to the mineralization of their residue. Higher significant adsorbable Phosphorus in the surface soil of the Bavanat site (semi-arid climate) can be due to its lower pH and, therefore, its higher availability in the soil. Moreover, significantly higher soil $CaCo_3$ in the Mehriz site (arid climate) can explain the significantly lower adsorbable Phosphorus of this site because, in the soils with high pH, adsorbable Phosphorus reduces in the presence of $CaCo_3$ [38].

There was a significant correlation between soil total Nitrogen and total Alkaloids of *C. ispahanica*. The concentration of Alkaloids usually increases with increasing soil total Nitrogen; Nitrogen is an essential element for the biosynthesis of these compounds [39]. Also, increasing total Phenolic and Flavonoid content along with antioxidant activity in *C. ispahanica* is correlated with soil Nitrogen content, and it is in concordance with previous studies [24]. Soil pH affects the availability of soil elements for plants [40], so it can also affect the production of SMs. This can explain a significant negative correlation between soil pH and the total Phenol and Flavonoid content of *C. ispahanica.*

Conclusion

It was concluded that heavier soil texture, semi-arid climate, and harvesting in the flowering stage are more suitable for *C. ispahanica* secondary metabolites production.

Acknowledgments

None declared by Authors

Ethical Permission: None declared by Authors

Authors' Contribution: None declared by Authors

Conflicts of Interest: The authors declare no conflict of interest.

Funding: This research received no specific grant from public, commercial, or not-for-profit funding agencies.

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[DOR: 20.1001.1.23222700.2023.11.2.6.8]
[DOI: 10.22034/ecopersia.11.2.93]