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ORIGINAL ARTICLE

Effects of Altitude on Some Physiological Characteristics of Sagebrush in Khorasan Province, Iran

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KEYWORDS

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ABSTRACT: The present research aimed to investigate the effects of altitude on some physiological characteristics of *Artemisia aucheri* Boiss. Plant samples were taken from 15 areas in the Lakh Kuhik mountains. After determining the maps of work units, samples were taken systematically in each unit. Data were analyzed using descriptive statistics in SPSS. The highest and the lowest chlorophyll content were recorded at an altitude below 1200 m and above 1256 m, respectively. Dry and fresh weights were measured with a precision balance. The results indicated a direct relationship between the increase in plant Ca^{2+}, Mg^{2+}, and K^{+} contents and altitude. However, there was no significant relationship between altitude and plant P^{3-}. Extraction was performed using a rotary evaporator (rotovap). Physiological and antioxidant traits and chlorophyll content of the samples were determined using the standard method (i.e., by a spectrophotometer). Results indicated that altitude had significant effects on physiological traits as the highest antioxidant activity was observed in regions with mid-latitude regions (i.e., 1228 m). Overall, it can be concluded that antioxidant content was lower at higher altitudes, and the best quality could be obtained from higher altitude plants (i.e., 1256 m). Similarly, fresh and dry weights were higher at higher altitudes because cytokinin levels in plants increased with reductions in auxin levels. In the next step, chlorophyll a and chlorophyll b were assayed separately. The results revealed that the chlorophyll content declined at high altitudes due to the presence of shortwave radiation.

INTRODUCTION

Ecophysiology refers to the study of interactions between living organisms and their surrounding environment. This science expresses the reactions of living organisms to the dominant and general conditions in the environment and investigates physical and physiological processes and ecological factors in living organisms. In addition, ecophysiology can describe the causal relationships (processes) between living organisms and their surrounding environment [1].

Linnaeus (1753) referred to the genus Artemisia for the first time in his Species Plantarum. Also, Cronquist (1968) determined its taxonomy as follows: class Magnoliopsida, subclass Asteridae, order Asterales, family Asteraceae, tribe Anthemideae, and Genus Artemisia.

Sumerians were the first who used plants for therapeutic purposes about 5000 years ago [2]. Medicinal plants have played a prominent role in human health and quality of life for thousands of years. Given the medicinal properties of their natural compounds, people have been utilizing their essential oils and extracts for a long time to treat various diseases. Therefore, these compounds have changed the medical systems of communities [3, 4]. According to the World Health Organization (WHO), about 80% of people in Asia and Africa depend on traditional medicine and medicinal plants to maintain their health. This treatment type relies mainly on using herbal extracts and their active compounds [5]. In

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addition, these plants play an important role in developed countries' healthcare systems. It has been proved that herbal medicines are important in treating various illnesses such as asthma, Parkinson's disease, cancer, ulcer, rheumatism, hypertension, malaria, and microbial, digestive, and respiratory diseases [6]. Therefore, modern communities resort to medicinal plants, including sagebrush, that enjoys numerous properties. As a member of the Asteraceae family, this plant is a shrub with extensive branches and many linear leaves. In another study, two-stage ohmic hydro-extraction was used to extract *Artemisia aucheri* Boiss essential oil, and the antioxidant and antimicrobial properties of the oil were investigated [7].

The effect of photosynthesis on life and Earth is crucial, and chlorophyll pigments play a key role in this process [8].

Pharmacological properties of sagebrush

Sagebrush has anthelmintic, antiflatulent, antitussive, anti-headache, disinfectant, appetizing, and antipyretic properties and acts as an analgesic for visceral pain. It is also used to treat hepatitis and as an insecticide. Sagebrush extract has antispasmodic effects because it blocks calcium channels and helps bronchodilation [9].

Secondary metabolites

A broad spectrum of organic compounds, called secondary metabolites, is produced in small quantities in the plant through the biosynthesis of primary metabolites. Secondary metabolites are found in special tissues and specific developmental stages. Also, they take part in plant defense reactions as an important physiological role. The plant molecules react with the sun's rays for less than 10^{-10} seconds and become excited, resulting in several highly active ions and free radicals [10 , 11]. These active oxygen species enhance the antioxidant system, including phenolic compounds comprising tannins, flavonoids, lignins, and simple phenols [12]. Environmental stress increases the production of secondary metabolites in plants. Therefore, plants growing at mountain heights are exposed to more severe stress caused by drought, sunlight, and ultraviolet light. Hence, they produce greater amounts of active ingredients than plants growing at lower altitudes. Altitude is among the essential and influential factors in plant growth and development. Temperature changes with altitude rise are among the most important factors affecting the changes related to altitudes of plant habitats. The explanation is that an increase or decrease in altitude can change factors such as temperature, relative humidity, wind speed, available water, and even solar radiation received[13].

Various species of Artemisia produce a broad spectrum of biologically active compounds, such as different terpenes and phenolic compounds, which have allelopathic properties [14 , 15]. These compounds include artemisinin, coumarin, camphor, borneol acetate, and cineol [16].

The diphenylpicrylhydrazyl (DPPH) radical suppression capacity is used as an important factor in studying antioxidative activity [17]. Phenolic compounds are also effective hydrogen donors and act as effective antioxidants [18]. In recent years, disease controlling capacity of plant antioxidants has been extensively studied. For example, the phytochemistry of sagebrush was investigated in a study on the levels of secondary metabolites at different altitudes. This research showed that sagebrush metabolites increase considerably at high altitudes. It also revealed the effect of habitat on secondary compounds in licorice. It was found that the root cortex of the plants highly contributed to antioxidant properties at high altitudes [19]. In an experiment on thyme essential oil in three regions of Hamadan, climatic conditions and soil type were reported to influence the essential oil content [20]. Climatic conditions affect the phytochemical properties of medicinal plants. In this respect, biomimicry is necessary for growing and domesticating medicinal plants, for which there is now an urgent need. The present research was conducted to compare the phytochemical properties of sagebrush in different habitats and to determine the best condition for growing this plant.

MATERIALS AND METHODS

Sampling

Samples of sagebrush plants were taken in three replicates from the heights of the Kuhak Mountains in

Karimabad Village of Roshtkhar County in Razavi Khorasan Province, located at 59°33′E and 34°50′N. The plants were identified in the Biology Department of Azad University of Damghan in the spring of 2018. This research was based on a factorial experiment that used a completely randomized design. The altitude and geographical coordinates of the habitats were determined and recorded by GPS. The type of mountainside was also specified. The lowest and the highest sampling altitudes on the mountainside were 1200 m and 1256 m, respectively (Medium altitude 1228). The desired biochemical traits were evaluated by collecting 18 samples from different altitudes.

Fresh and dry weights

The fresh samples were weighed, and the aerial organs, including leaves (for 48 h) and stems (for 72 h), was oven-dried at 80°C and weighed with a precision balance.

Methanolic extract

The collected samples were dried at 20°C in the shade with proper ventilation. They were then ground, and 100 *g* of each ground sample was poured into a 1-*L* Erlenmeyer flask, to which 1 *L* methanol was added. The flask was put in a water bath at 37°C for 48 h. The samples were purified using a vacuum pump, extraction was performed using a rotary evaporator, and the extract was used to assess the antioxidants.

Antioxidant assessment

The method introduced by Ebrahimzadeh *et al.* (2008) was used for the antioxidant assay. To this end, 40 *mg* of the extract was dissolved in 25 *mL* methanol. The stable radical DPPH at 4 *mg*/100 *mL* (0.1 *mM*) was dissolved in 100 *mL* methanol. Next, five concentrations of each extract were prepared. For this purpose, solutions with concentrations of 12.5 to 500 $\mu g/mL$ of each extract were dissolved in methanol. Then, 2 *mL* of each prepared concentration was poured into a 2-*mL* test tube, to which 2 *mL* DDPH was added. The tubes were put in the dark for 15 min, and absorbance was read at 517 *nm*. The obtained values were converted into radical scavenging activity (RSA) using the following relation:

$$\text{Radical scavenging activity (DPPH)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} represents the absorbance of the control solution at 517 *nm* and A_{sample} the absorbance of the sample at 517 *nm*.

Chlorophyll assay

Plant leaves (0.2 *g*) were ground in a porcelain mortar with 10 *mL* of 80% acetone, the mixture was centrifuged at 4800 *g* for 20 *min*, and the extract was separated. The final volume of the extract was raised to 20 *mL* by adding 10 *mL* of 80% acetone. Absorbance was read at 645 and 633 *nm* using a spectrophotometer to calculate quantities of chlorophylls a and b. Finally, their quantities were assessed in *mg* using the fresh weight of each sample [21].

RESULTS

Identifying various habitats, evaluating environmental factors' effects on morphological traits, and assessing qualitative and quantitative yields of secondary metabolites in medicinal plants help considerably domesticate these plants and maintain their genetic diversity [22].

Calcium and Magnesium levels

Tables 1 and 2 lists the coefficient of determination (R^2) values between the independent variables (Ca^{2+} and Mg^{2+}) and the dependent variable (plant height). Since the R^2 for this variable was 0.974, there was a very strong correlation between the two variables of Ca^{2+} and Mg^{2+} and plant height. The significance level was also 0.00, which confirms the significance of this relationship (Figure 1).

Table 1. Effect of altitude on concentration of plant elements.

		(K%)	(p%)	Ca,mg (%)
	1200	1.5	0.141	1.51
	1204	1.5	0.149	1.52
	1208	1.6	0.147	1.55
	1212	1.65	0.145	1.57
	1216	1.71	0.136	1.65
	1220	1.8	0.122	1.74
	1224	1.8	0.108	1.83
Height	1228	1.82	0.130	2.3
	1232	1.86	0.150	2.17
	1236	1.89	0.151	2.23
	1240	1.92	0.152	2.31
	1244	1.98	0.154	2.45
	1248	2.1	0.157	2.56
	1252	2.3	0.160	2.61
	1256	2.37	0.168	2.7

Table 2. Plant Regression Analysis.

Dependent variable	Element	R^2	t-value	Sig.
	K	0.93	13.186	0.000
Height	p	0.255	28.267	0.055
	Ca,mg	0.974	299.603	0.000

Phosphorus level

The R^2 values between the independent variable (P^{3-}) and the dependent variable (plant height) are presented in Table 1. Since the coefficient of determination for this variable was 0.255, there was a very weak correlation between the two variables of P and plant height. The level of significance (Sig) was also 0.055, which confirms this relationship was not significant (Figure 1).

Potassium (K^+) level

Tables 1 and 2 shows the coefficient of correlation (R Square) between the independent variable (K^+) and the dependent variable (plant height). Since the coefficient of determination for this variable was 0.930, there was a very strong correlation between the two variables of K^+ and plant height. The significance level (sig) was also 0, confirming the significance of this relationship (Figure 1).

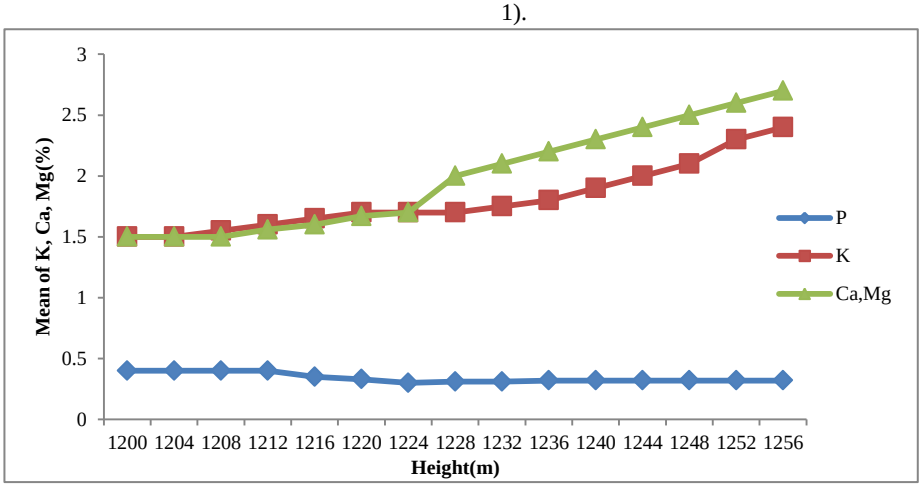


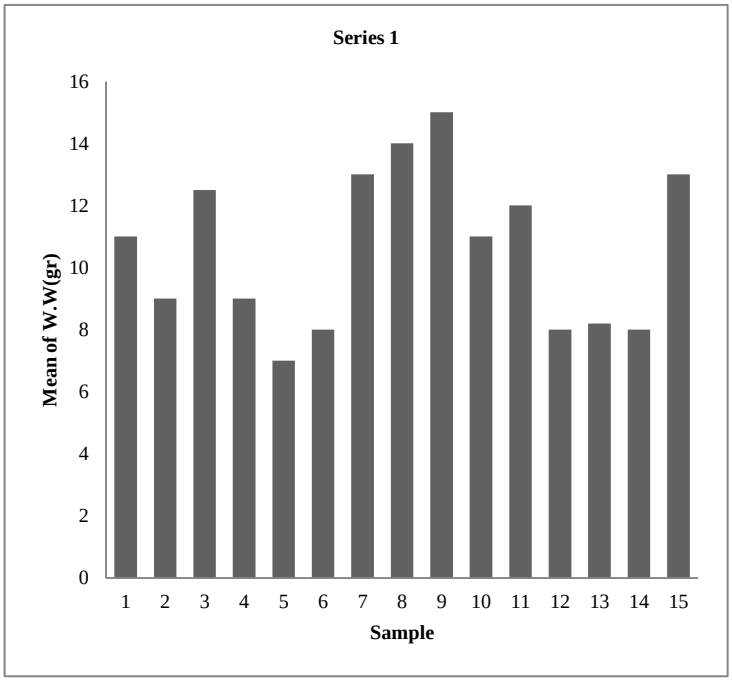
Figure 1. Effect of altitude on concentration of plant elements

Fresh and dry weights

According to the descriptive statistics and the related table, the highest weight was 15.38 for sample 17, while the lowest for sample 5 was 7.24. The standard deviation of the data is 2.53, the variance is 6.43, and the mean is 11.51 (Figure 2).

According to the descriptive statistics and the related table, the highest weight was 14.94 for sample 17, and the lowest for sample 5 was 6.94. The standard deviation of the data is 2.56, the variance is 6.55, and the mean is 10.8 (Figure 3).

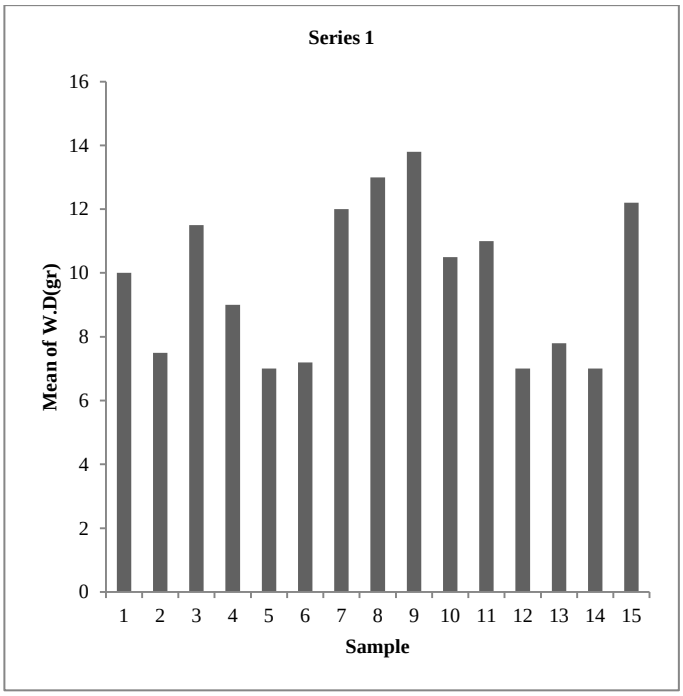
Fresh and dry weights increased in the sunlight-exposed plants. This enhanced growth of young and dividing meristematic cells was probably caused by improved cell division [23]. Certainly, the increase in growth and cell division in the sunlight-exposed plants may result from intracellular changes in the ratio of cytokinin to auxin. Since cytokinin plays an essential role in cell division, it can affect the cell cycle progression at M to G1 and S to G2 [24]. The auxin level in plants is an essential physiological factor for their growth. Available evidence shows that auxin declines following the exposure of the plant to sunlight. This result explains the observed increase in fresh and dry weights at higher altitudes.



More numbers

N	Valid	18
	Missing	0
Mean		11.3007
Std. Error of Mean		.59781
Median		11.5150
Mode		7.24[a]
Std. Deviation		2.53628
Variance		6.433
Skewness		.096
Std. Error of Skewness		.536
Kurtosis		-1.287
Std. Error of Kurtosis		1.038
Range		8.14
Minimum		7.24
Maximum		15.38
Sum		203.41

Figure 2. Diagram of fresh weight (W.W)



Dry numbers

N	Valid	18
	Missing	0
Mean		10.8011
Std. Error of Mean		.60304
Median		11.0783
Mode		6.94[a]
Std. Deviation		2.55849
Variance		6.546
Skewness		.101
Std. Error of Skewness		.536
Kurtosis		-1.258
Std. Error of Kurtosis		1.038
Range		8.00
Minimum		6.94
Maximum		14.94
Sum		194.42

Figure 3. Diagram of dry weight (W.D)

Chlorophyll

Chlorophyll content at various altitudes

Since Sample 1 was taken at the lowest altitude (1200 m), it had the highest chlorophyll content. In addition, the lowest chlorophyll content was related to Sample 15, taken at the highest altitude (1256 m) (Fig. 4).

Since solar radiation contains different wavelengths, UV rays can damage the reaction center in photosynthesis II. Therefore, they can inhibit ion accumulation in leaves, increase the activity of the chlorophyll degrading enzyme (chlorophyllase), reduce chlorophyll synthesis, and lower the resistance of the thylakoid membrane [25]. Chlorophyll concentration decreases at higher altitudes, and old leaves turn yellow and drop after a long period of stress. Han and Lee (2005) attributed the reduction in chlorophyll content during stress conditions to the increased degradation or reduced production of these pigments and the inactivity of their synthesizing enzymes. In the present research, chlorophyll decreased at high altitudes and reached a maximum at low altitudes (Figure 4).

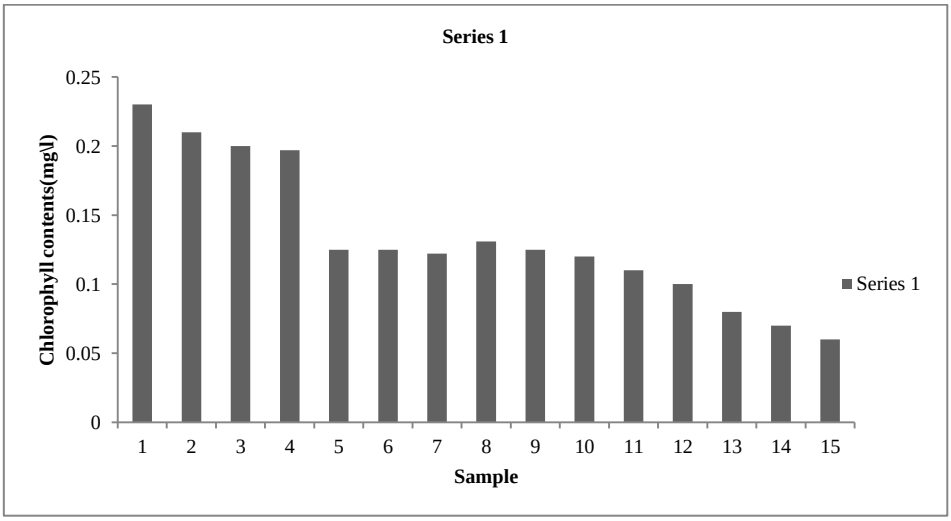


Figure 4. Chlorophyll contents at various altitudes

Antioxidants

Most physiological stresses can disrupt the plant metabolism and cause oxidative damage through ROS production [26]. Since plants have limited mechanisms for avoiding stress, they adapt themselves to variable environmental conditions.

Plants and other organisms have developed a broad spectrum of antioxidant defense mechanisms against ROS. Activities and capacities of antioxidant defense mechanisms in plants, including antioxidant compounds and enzymes, limit oxidative damages and destroy ROS. Plants have expanded their antioxidant systems to prevent the excessive production of reactive molecules [27]. In response to ROS, cells use their antioxidant defense mechanisms, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), regarding their important roles in defending plants against ROS-induced damages. Plants have complicated antioxidant systems to escape from the harmful effects of ROS. Environmental stresses, including UV rays, increase ROS production, which results in the oxidation of photosynthesis pigments, membrane lipids, proteins, and nucleic acids. The present research showed that antioxidants were of high at mid-altitude types.

Characteristics of habitats and the status of plants in nature can greatly influence the number of essential oils and active ingredients in plants. Reports have shown a relationship between habitat conditions and chemical compounds in plants and a basic relationship between the geographical origins of plants and their effective compounds [28].

DISCUSSION

A different growth pattern was observed in the aerial parts of cultured plants under gamma radiation and exposure to light radiation (i.e., red, yellow, blue, or white). WW, leaf area, and shoot length decreased under the gamma rays irradiated at 15-45 Gy. The shoot was found to grow significantly under single-color light radiation. Adding gamma radiation, in addition to red and yellow light treatments, significantly increased chlorophyll content in stems and leaves. Interestingly, the leaf area under the yellow light treatment at 15-100 Gy was significantly higher than that of the red light at the same dose [29]. The production of frangible, thick, and tough leaves occurs due to high levels of gamma irradiation. The size of the upper epidermal and the sponge and palisade parenchyma depends on the gamma irradiation intensity [30]. Marud and Greenberg (1996) reported that gamma radiation destroyed grana and stroma thylakoid [31].

Long-term and severe solar radiation may lead to singlet oxygen generation that can harm the photosynthetic apparatus. Plants have several mechanisms to deal with intense radiation and singlet oxygen generation to protect chlorophyll against them [32]. The main effect of radiation on cell membranes is lipid peroxidation by free radicals [33].

Higher salinity levels lower fresh weight but slightly affect the amount of lycopene synthesis and chlorophyll degradation [34]. Photosynthesis plays a vital role in life and Earth. In this process, chlorophyll pigments play a key role [35]. Furthermore, chlorophyll plays an essential role in human nutrition. For instance, the chlorophyll existing in green vegetables can prevent the development of chronic diseases due to its biological functions. Besides, chlorophyll, in addition to its antioxidant activity, can prevent cancers and gene mutations. Today, much attention is paid to chlorophyll and its derivatives due to their anti-cancer effects [36]. When stresses such as salinity affect plants, their ROS level naturally increases as a natural mechanism [37]. Research has shown that the key steps of chlorophyll biosynthesis are prevented by oxidative stress [38]. Chlorophyll concentration decreases at high altitudes, and old leaves turn yellow and fall after a long period of stress. The present research showed that chlorophyll content decreased at high altitudes, and its highest content was observed at low altitudes (Figure 4).

The Olsen method was employed in this study for phosphorus extraction. This method, which has shown a good correlation with plant responses under aerobic and anaerobic conditions, has been used in many studies on a wide range of soils for predicting the phosphorus available to plants [39]. In this method, bicarbonate and hydroxyl act as two rivals in separating phosphorus from soil particles. It is a more appropriate choice than other

methods because of its short extraction duration and suitability for all soil types. Several scientific studies have investigated the mineral content of several different plants (*Artemisia, Ipomoea aquatica, Astragalus, Acanthophyllum, Asystasia gangetica, Enhydra fluctuans,* and *Oldenlandia corymbosa)* [40,41, 42].

Conflict of Interest

The authors declare no conflict of interest.

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