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Ecological Properties of the Medicinal Plant-*Helleborus orientalis* Lam.

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Abstract: In this study, nutrient concentrations (N, P and K) in above and below ground parts of *Helleborus orientalis* Lam. which has several medicinal properties were examined during vegetative and generative growth periods. There were significant differences between two growth periods in different parts of *H. orientalis* in terms of N and K concentrations. We have only found statistically significant differences between the studied localities, in terms of K concentrations. There were no significant differences with respect to soil factors except K and organic matter concentrations of the studied localities. Our findings are supported the vernal dam hypothesis.

Key words: *Helleborus orientalis* Lam., macro-element concentration, medicinal plants, growth periods, vernal dam hypothesis

INTRODUCTION

The ecological success of a species depends that how it adapt to a particular physical environment and establish biological relations with other species[1]. The life span of species which have a great economic value is very important to clarify. Economic plants are used for flavoring foods and beverages, medicines, cosmetics, dyes and perfumes, for other household. Because of such useful properties economic plants are continuously disturbed and tubers are exported illegally[2].

Medicinal plants are economic plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of medicinal plants in most developing countries serves as a basic for the maintenance of good health[3]. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, more than 30% of the pharmaceutical preparations are based on plants in the world[4]. The use of medicines from plants in the form of local medicine dates back 4000-5000 B.C. While the medicinal values of these plants are due to presence of small doses of active compounds which produces physiological actions in the human and animal body[5]. Some of the important bioactive compounds found in medicinal plants are alkaloids, glucosides, resins, gums, musilages etc.[6].

Naturally, in terms of economic plants, Turkey, is one of the richest countries of Europe and the Middle East because of its climate and geographical position. However, no much attempt has been made to work and utilize natural resources of this country. *Helleborus orientalis* is one of these species which has economic value and possesses medicinal properties.

H. orientalis Lam. is a herbaceous plant usually prefers forest clearings. It also has several important medicinal properties. *H. orientalis* probably possesses similar medicinal virtues as well as do the roots of some other species of the same genus. In the ancient times this species was an appreciated as a medicinal plant with miraculous curative effects especially on a mental disorders.

H. orientalis occurs in the Western, Central and East Black Sea Region, on the North of Turkey. This species is belonging euxine sector of the Euro-Siberian floristic region phytogeographically. It is also native for Georgia Republic and Caucasus. This species is locally known as Çöpleme, Bohçaotu, Danabağırtan in Turkey.

Herbaceous plants occur on forest clearings should have carried out their primary functions (i.e. nutrient transfer between different parts) prior to canopy closure[7]. They usually evade stress conditions such as shade, drought etc. by survival in below ground tissues and they exhibit, both the features of sun and shade plants[8].

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In this study, distribution of some macroelements (N, P, K) among different parts of *H. orientalis* and some ecological soil properties during vegetative and generative growth periods were investigated. These results can be considered for cultivation this species.

MATERIALS AND METHODS

Study area: Plant samples were collected during 1999-2000 years from Engiz (41°35′N; 35°56′E), Trabzon (41°00′N; 38°43′E), Istanbul (Belgrad Forest: 41°09′N; 29°03′E), Bafra (Elifliköy: 41°35′N; 35°56′E), Samsun (41°17′N; 36°20′E) from Black Sea Region of Turkey. Engiz, Trabzon, Istanbul, Bafra and Samsun populations were collected at 30, 50, 100, 200 and 300 m, respectively.

Mean annual temperature and mean annual precipitation in Engiz are 13.5°C and 672.4 mm, respectively. Mean annual temperature in Trabzon and Istanbul is 14.6 and 12.8°C, respectively. Mean annual rainfall in Trabzon and Istanbul is 822.7 and 1074 mm, respectively. Mean annual temperature in Bafra and Samsun is 14.3 and 14.4°C, respectively. Mean annual precipitation in Bafra and Samsun is 712 and 735 mm, respectively[9].

The study area is situated between A_2 (E) and A_7 squares according to the grid systeme of Davis[10].

Five plant individuals were used for macroelement analysis in each of vegetative and generative growth phases. Phenological observations were also recorded.

Chemical analysis: Plant samples were harvested during vegetative that is in 2nd half of April and generative that is in second half of December growth phases and separated into root, shoot, leaf and flower parts. Nitrogen (%) was determined by the micro Kjeldahl method with a Kjeltec 1030 Analyser (Tecator, Sweden) after digesting the samples is concentrated H_2SO_4 with a selenium catalyst. For P (%) and K (%) analysis plant specimens were wet ashed in concentrated HNO_3 and $HClO_4$ and P was determined by using Jenway spectrophotometer and K was determined by Petracourt PFP-7 flame photometer after nitric acid wet digestion[11].

Soil samples taken from a depth of 0-30 cm, were also collected during vegetative and generative growth phases separately and soil and plant samples were taken simultaneously during these phases. Soil texture was determined by Bouyoucus hydrometer method. Soil nitrogen (%) was determined by micro Kjeldahl method. Soil phosphorus (%) was determined spectrophotometrically following the extraction by ammonium acetate. Soil potassium (%) was determined by using a Petracourt PFP-7 flame photometer after nitric acid wet digestion. Organic matter (%) concentration was determined by Walkley-Black method[12]. The results of soil analysis were explained according to Pirdal[1].

Statistical analyses were performed using MINITAB software package[13].

RESULTS

Phenological observations: The duration of vegetative growth period in *H. orientalis* is between at the end of April and at the end of November. Flowering begins at the middle of December and continues up to February. At the first half of March fruit ripening is occurred. The time interval for seed dispersion is between at the end of March to the end of April. In Trabzon samples generative growth phase is lasted one month later as compared to the other regions.

Plant and soil analysis: *H. orientalis* occurs on clay-loamy and clay-sandy-loamy soils. During vegetative growth phase, the highest N (%) concentration was observed in leaves of *H. orientalis*. and the lowest N (%) concentration was observed in shoots during vegetative growth stage. P (%) content of leaves was also higher as compared to shoots and roots. The highest K (%) concentration was found in shoots and leaves in vegetative growth phase.

In generative growth period, higher concentration of N (%) and P (%) was observed in flowers and leaves as compared to roots ans shoots. Plants growing in Engiz and Istanbul show the highest P (%) concentration was found in roots. K (%) concentration was higher in shoots and flowers. The lowest K (%) content was observed in roots during generative growth phase.

There were significant differences among the different parts as macroelement concentration in both vegetative and generative growth phases (Table 1) except P concentration in *H. orientalis* which is non-significant. There were also significant differences in respect of

Table 1: Comparison of N, P and K (%) concentration in different parts of *H. orientalis* by one-way ANOVA test during vegetative and generative phases

Growth phase	Nutrient	F-value	p-value	S
Vegetative	N	19.403	0.000	**
Generative	N	6.871	0.000	**
Vegetative	P	0.869	0.425	NS
Generative	P	1.004	0.396	NS
Vegetative	K	7.092	0.002	**
Generative	K	7.958	0.000	**

p: Probability, *p<0.05, **p<0.01, NS: Not Significant

Table 2: The comparison of macroelement concentrations in roots of *H. orientalis* in respect of growth phases and localities by two-way ANOVA

E	Source	df	Sum of squeres	Mean square	F-value	p-value
N	Growth phase	1	0.016	0.016	0.04	0.087 NS
N	Locality	4	3.380	0.845	2.03	0.115 NS
N	Error	30	12.472	0.416		
P	Growth phase	1	0.00529	0.00529	1.47	0.235 NS
P	Locality	4	0.01450	0.00362	1.01	0.420 NS
P	Error	30	0.10805	0.00360		
K	Growth phase	1	0.000	0.000	0.00	0.992 NS
K	Locality	4	1.0462	0.2615	3.77	0.013 *
K	Error	30	2.0820	0.0694		

E: Elements, *p<0.05, NS: Not Significant, df: Degrees of freedom, p: Probability

Table 3: The comparison of macroelement concentrations in shoots of *H. orientalis* in respect to growth phases and localities by two-way ANOVA

E	Source	df	Sum of squeres	Mean square	F-value	p-value
N	Growth phase	1	0.0196	0.096	0.60	0.444 NS
N	Locality	4	1.005	0.251	1.57	0.207 NS
N	Error	30	4.794	0.160		
P	Growth phase	1	0.008237	0.008237	15.70	0.000**
P	Locality	4	0.005595	0.001399	2.67	0.051 NS
P	Error	30	0.015736	0.000525		
K	Growth phase	1	0.0000	0.0000	0.00	0.992 NS
K	Locality	4	1.0462	0.2615	3.77	0.013*
K	Error	30	2.0820	0.0694		

E: Elements, df: Degrees of freedom, p: Probability, *p<0.05, **p<0.01, NS: Not Significant

Table 4: The comparison of macroelement concentrations in leaves of *H. orientalis* in respect to growth phases and localities by two-way ANOVA

E	Source	df	Sum of squeres	Mean square	F-value	p-value
N	Growth phase	1	0.188	0.188	0.46	0.503 NS
N	Locality	4	1.605	0.401	0.98	0.434 NS
N	Error	30	12.295	0.410		
P	Growth phase	1	0.0833	0.0833	8.15	0.008**
P	Locality	4	0.0503	0.0126	1.23	0.139 NS
P	Error	30	0.3066	0.0102		
K	Growth phase	1	0.3250	0.3250	10.56	0.003**
K	Locality	4	0.5198	0.1300	4.22	0.008**
K	Error	30	0.9236	0.0308		

E: Elements, df: Degrees of freedom, p: Probability, *p<0.05, **p<0.01, N.S: Not Significant

Table 5: The comparison of macroelement concentrations in flowers of *H. orientalis* by one-way ANOVA according to different localities

Elements	F-value	p-value	Significance
N	3.967	0.022	*
P	2.725	0.069	NS
K	0.766	0.563	NS

*p<0.05, NS: Not Significant

Table 6: The comparison of N, P, K (%) and organic matter concentrations in soils in respect to growth phases and localities by two- way ANOVA

E	Source	df	Sum of squeres	Mean square	F-value	p-value
P	Growth phase	1	0.000854	0.000854	4.29	0.052 NS
P	Locality	4	0.001394	0.000348	1.75	0.179 NS
P	Error	20	0.003981	0.000199		
N	Growth phase	1	0.007	0.007	0.01	0.924 NS
N	Locality	4	3.280	0.820	1.16	0.359 NS
N	Error	20	14.177	0.709		
K	Growth phase	1	0.0198	0.0198	0.87	0.361 NS
K	Locality	4	1.3664	0.3416	15.02	0.000**
K	Error	20	0.4549	0.0227		
OM	Growth phase	1	0.356	0.356	1.38	0.254 NS
OM	Locality	4	4.217	1.054	4.08	0.014*
OM	Error	20	5.169	0.258		

**p<0.01, *p<0.05, NS: Not Significant, E: Elements, df: Degrees of freedom, p: Probability, N: Nitrogen, P: Phosphorus, K: Potassium, OM: Organic Matter

growth phases and localities in both plant and soil samples (Table 2-6).

DISCUSSION

The highest macroelement concentration was generally found in leaves during vegetative stage (Table 7). The main reason of the high nutrient contents in leaves during vegetative growth period was due to intensive physiologic activities of above ground parts in this stage and transportation of nutrient elements to the above ground parts, especially to the leaves. Kutbay and Kılınç[14] have pointed out that the same circumstance was present in *Ferula communis* (L.) (Umbelliferae). In

Table 7: Mean nutrient contents±standard error in different parts of *H. orientalis*

	N				P			
	Root	Shoot	Leaf	Flower	Root	Shoot	Leaf	Flower
Vegetative phase	2.100±0.371	1.545±0.250	2.769±0.207	-	0.066±0.019	0.053±0.014	0.121±0.012	-
Generative phase	2.062±0.206	1.628±0.172	2.634±0.360	2.772±0.454	0.044±0.024	0.026±0.006	0.031±0.008	0.044±0.01

	K			
	Root	Shoot	Leaf	Flower
Vegetative phase	0.269±0.075	0.557±0.093	0.592±0.088	-
Generative phase	0.194±0.05	0.532±0.148	0.360±0.076	0.418±0.102

Table 8: Mean nutrient contents±standard error in soil samples during growth phases

	N	P	K	Organic matter
Vegetative phase	1.684±0.533	0.011±0.005	0.543±0.84	4.357±0.208
Generative phase	1.654±0.346	0.021±0.007	0.523±0.059	4.575±0.275

addition, in vegetative stage division of meristematic cells in above ground parts is fast. High nitrogen contents in meristematic tissues were depend on the high protein content of that tissues[15,16]. Anderson and Eickmeier [7] stated according to vernal dam hypothesis forest herbs or herbs distributed around forest clearings temporarely sequester nutrients in deciduous forests prior to canopy closure and return them to the below ground tissues following senescence of above ground tissues. This phenomena is also known as top senescence. In top senescing plants (i.e. geophytes) new shoots appear at the beginning of the next season and above ground tissues senesce completely during that period[17,18]. There were significant differences among different parts of *H. orientalis* from the stand point of nitrogen and potassium during vegetative period. However, there were no significant differences as phosphorus concentration. This is related to the phloem mobility of the nutrients. N, P and K can be ranked as K>N>P in respect to phloem mobility.

In other words, P is a phloem-immobile nutrient as compared to K and N[19].

It was observed that flowers of this species contained higher concentration of macroelements in generative stage. This is arise from translocation of macroelements to the flowers and accumulation of nutrients as a pool in reproductive organs[17]. There were also established significant differences among the different parts as N and K during generative growth phase. There were no significant differences among the different parts as P concentration during generative growth stage.

H. orientalis occurs on the soils that have high nitrogen (%) concentrations. K (%) concentration was usually at medium levels[1]. However, P (%) concentrations were at low, medium and high levels[1]. This species occurs on the soils that have high organic matter (%) in respect to different localities (Table 8). There was significant differences as soil organic matter in respect of different localities. This is related to much annual precipitation and soil temperature. Soil organic matter has been rapidly decomposed when annual rainfall and temperature is high. Additionally, soil organic matter content has been affected by plant density. There was also determined significance differences as soil K with respect to localities. K concentration decreases by leaching in soils where annual rainfall is high. But in Istanbul K concentration of soils is high while annual precipitation is 1074 mm in this region. The main reason of the high K concentration in soil samples collected from Istanbul (Bahçeköy) due to dominance of illit (hydromicaceous) clay type in this region[20]. In soils with illit type clays, for balancing negative ions, potassium does not decrease by leaching in spite of high precipitation.

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