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ORIGINAL ARTICLE

Survive *Leucojum aestivum* by study growth conditions in three different regions

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ABSTRACT
Leucojum aestivum L. is a threatened plant species that is currently used as a commercial source of Galanthamine, bulbs are full of alkaloids used as in medicine. The species can grow in 1000 m altitude and found in the western mediterranean region. In order to evaluate some traits of summer snowflake in three different regions of the north of Iran (Langrood, Lahijan, Tonekabon), to show the effect of natural habitats on plant growth, plants were collected at the full flowering stage in 2017. This experiment were designed in random plots in complete randomize with three replications. The size of plots was 25 m², and 9 plants in each plot were evaluated. Flower stem length, flower diameter, leaf number, leaf length, leaf width, leaf area and flower number were recorded. The weight of leaves of plant, flowers of plant, stem and bulb of plants were measured and then conclusion of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu in leaves were determined. The results of variance analysis showed that there was a significant difference between habitats on flowering stem length, flower stem weight, percent of magnessium and concentration amount of cupper (α≤0.05). Also there were significant difference between habitats on leaf length, leaf width, leaf surface, large leaf weight, total leaf weight, bulb weight, plant weight, percent of potassium and amount of magnesium (α≤0.01). It was observed that the maximum leaf long belong to Lahijan plants, so the highest leaf weight with 3.56 g/per plant, bulb weight (20.85g/per plant), plant weight (38.8g/per plant), manganese (27.7 mg/kg) was related to the Langrood plants. The result indicated the effect of organic matter and amount of elements on plant growth had positive effect.
Keywords: *summer snowflake, habitat, growth, survive*

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INTRODUCTION

Leucojum aestivum L. (Amaryllidaceae), known as summer snowflake, is a threatened plant species that is currently used as a commercial source of Galantamine. It is gathered from the natural habitats for industrial purposes, this causes increasing problems with depletion of the wild populations [3, 7]. Most of the Leucojum species are found in the western Mediterranean region. *L. aestivum* is a species whose distribution in the north Ireland, Czech and Slovakia, and in the east Turkey, the Crimea and Iran [20, 6]. The species can grow in 1000 m altitude [17]. From the eastern to the western limit of its range, *L. aestivum* subsp. aestivum occurs south of the Caspian Sea, in northern Iran [12]. The genus Leucojum consists of 11 species. *L. aestivum* is the only species of the subgenus Aerosperma [4].

L. aestivum is a perennial bulbous geophyte. The bulb is sub-spherical (up to 6 cm in diameter), with a brown tunic [20], which helps the plant to survive in the summer dry period; it contains the basal plate, fleshy scales, 1–4 shoots, and sometimes lateral buds. Adult bulbs were found to be 10.7±1.2cm below the soil surface. The bulb shows a sympodial branching system, each unit of which is composed of 6–8 foliage leaves, and a lingulate scale, and terminates in an in florescence [14]. Each plant can produce up to 3 (4)

flowering scapes, but more commonly only one [20]. In its native distribution area *L. aestivum* subsp. aestivum occupies alluvial habitats near rivers, lakes or on the banks of canals, and humid, often periodically flooded sites, which are shaded, semi-shaded or in full light [8, 9, 16, 21]. The soil of the habitats is usually sedimentary, deep and compact, consisting of clay or loam, with a very fine texture and hydrophilic character [1, 19]. It is rich in nitrate and humus and poor in carbonates, with a moderately acid to slightly basic pH. *L. aestivum* occurs also far from water bodies, but only where a sufficient amount of water in the soil is available during the growing season from February to the end of May [15]. In its habitats, the plant generally occupies microdepressions, where soil moisture is higher [19]. Very little is known about the habitats in which *L. aestivum* grows. It occurs in regions characterized by warm to hot, dry summer sand mild, wet winters [5], and as an introduced plant also in regions with a temperate oceanic bio climate [18]. According to Guinea Lopez and Ceballos Jimenez [11] the species grows in Mediterranean wet grasslands and on river banks. In SE-France it occurs at altitudes of 0–100m and in Corsica of 0–300m [13].
The objective of the present study was to determine some traits of *L. aestivum* in three different regions, to show the effect of natural habitats (climate) on plant growth.

MATERIALS AND METHODS

In order to evaluate different traits of *Leucojum aestivum* L. (Amaryllidaceae) and evaluation effect of natural habitats (climate) on plants, were collected at the flowering stage during 2017, from three regions of the north of Iran:
1. Langrood, Guilan province,
2- Niakoo, Lahijan, Guilan province,
3- Miankooh, Khorramabad, Tonekabon, Mazandaran province.
Soil samples from sites were collected from the root area at 20 cm depth. In each site, six samples were collected within a plots in size of 25 m^{2}and combined to make a composite soil sample [10]. The characters of the soil of locations were showed in Table 1.

Table 1- soil characteristics of three studied regions

Location	pH	EC (ds/m)	O.C (%)	P (mg/kg)	K (mg/kg)	Sand (%)	Silt (%)	Clay (%)	Texture
1 Langrood	7.1	0.8	2.8	76.6	432	24	48	28	Loam
2. Lahijan	8.02	0.601	4.37	36.1	353.8	52	34	14	Loam
3. Tonekabon	8.60	0.888	4.60	12.20	184.6	44	30	26	Loam

Vahdati Soil Science Lab- Iran

In this experiment random plots were used in complete randomize design with three replications. The size of plots was 25 m^2, and 9 plants in each plot were studied. Flower stem length, flower diameter, leaf number, leaf length, leaf width, leaf area and flower number were recorded. The weight of small leaf, large leaf, total leaves of plant, main flower, total flowers of plant, flower, bulb and complete plant per plant were measured and then absorption content of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu in leaves was determined. The leaf samples were dried in oven at 70ºC for 24 hours.
Approximately 0.2 g of the dried leaf samples, were treated individually with 8 mL HNO3 (65% Merck) and 10mL H_2O_2 (30% Merck) and then were mineralized using a Berghof MWS-2 microwave digestion system. After 40 minutes of digestion, the samples were cooled for 30 minutes and the clear solutions were filtered and brought at 50mL with distilled deionized water. elements concentrations in the final solutions were analyzed by Flame Atomic Absorption Spectrophotometry (FAAS), using an AVANTA GBC spectrophotometer. Determination of elemental concentrations in leaves samples were performed using the method of curve calibration according to the absorber concentration. The element concentrations were reported as mg/kg dry weight [10]. Obtained data were tested for normality, and then randomized complete design was used for analyzing via SAS software.

RESULTS AND DISCUSSION

The results of variance analysis showed that there was a significant difference between habitats on flowering stem length, flower stem weight, percentage of Mg and Cu ($\alpha \leq 0.05$), also leaf length, leaf width, leaf surface, leaf weight, bulb weight, plant weight, percentage of K and Mn at 0.01% level (Table 2).
The results of mean comparisons showed that the minimum length of the flower stem (59.7 cm) and the maximum (69.9 cm) was observed in Langerood plants (region 2) and Lahijan (region 3), respectively. Table 1 shows that the soil organic matter of Langrood has been about half the others which can be

effective in the longitudinal growth of the flower stem as indicated in the research, that the soil of the habitats is usually sedimentary, deep and compact, consisting of clay or loam, with a very fine texture and hydrophilic character [1, 19]. The longest leaf with 66.7 cm was belonged to plants in the Lahijan. The largest leaf area with 449.87 cm^2/plant was belonged to Langrood. Considering the high rainfall in the north of Iran, it seems that the plants in this region have grown better due to the sandy soil of Lahijan.

The highest flower diameter was in Langrood (21.68cm) and Lahijan (19.91cm). The highest leaf width (18cm) was obtained from the Langrood. The highest weight of the largest leaf was obtained with 0.63 g from the Langrood. It was observed that the maximum leaf weight with 3.56 g/plant was related to the Langrood.

The highest bulb weight (20.85g), flower stem weight (14.3g/plant), plant weight (38.8g/plant), magnesium concentration content to plant (27.7 mg/kg) was in Langrood (Table 3).

Considering that the Langhorod site pH is less than others, so in acid soils the solubility of the elements increases and can be effective in increasing plant growth. Also, the soil of this region has more phosphorus and potassium than other regions. These elements are high consumption and can be very effective in growth and development of plants [15].

The highest potassium percent (2.2%) and percentage of magnesium (0.24%) was observed in Tonekaboon region plants. Due to the negative correlation between magnesium and potassium and low potassium content in Tonekabon soil (Table 1), this issue needs to be investigated.

The comparison of plants of different habitats is a suitable method for meeting some nutritional needs and optimal growth conditions of plants in order to investigate their compatibility in crop conditions, so that by choosing relatively similar places with natural habitat, the confidence level of success could be high [2].

The results of variance analysis showed that there was a statistically significant difference between three regions in terms of length of flowering stem, leaf length, leaf width, leaf weight, plant weight, bulb weight, shoot weight, potassium and magnesium percent, manganese and copper concentration content(Table 2).

Investigating the amount of soil elements in the three regions showed that there was a significant difference between the three regions in terms of available potassium, so that the potassium absorption percentage of Langrood was 432 ppm, while potassium content of Lahijan was 353.8 ppm and Tonekabon 184.6 ppm (Table 1). Therefore, the importance of soil examination is more likely to be found in potassium-absorbed in plants, because the results of the comparison of the average of potassium percentage showed that the amount of potassium absorbed by Tonekabon was more than two other habitats, which is also the absorption of copper in the herb habitat were in the first group.

It seems that the plant has a low need for these elements and may be dependent to relationship between elements, because there is a little knowledge about the habitats which *L. aestivum* grows.

Mean comparison of traits showed that the highest leaf weight, total leaf weight, bulb weight, flower stem weight and plant weight were related to Langrood region plants, that this results belong to soil conditions. The study of soil degradation of three habitats showed that Langrood soil was rich in phosphorus and potassium in comparison to the other two habitats, that shows the effect of this macro elements in bulb and plant weight (Table 2).

In neutral pH and in neutral conditions, the solubility of most of the elements of soil is high and its absorption capacity increases, and as a result, the positive effects of different elements such as nitrogen, iron and magnesium could be observed in the growth rate of plants.

In addition, the high percentage of minerals (N+P+K+Ca+Mg) with 4.9% indicates that the absorption and maintenance of water in the habitats soil was high and due to the loss of root in soil because of loamy texture, the possibility of growing more gens is provided.

Investigating the correlation between different elements in this plant shows that there is a very little positive or negative correlation between the elements (Table 4). There was a significant positive correlation between nitrogen and Manganese and there was no significant correlation with the other elements (Table 4). Potassium with magnesium, and calcium with iron, had a positive correlation while non-significant negative correlation was observed between most of the elements (Table 4).

Though non-significant, negative correlation is one of the characteristics of cultivated plants with low fertilizer requirements.

One of the interesting points in the correlation table was the significant positive correlation between zinc element and most of the other traits, indicating that special attention should be paid to the zinc element in agronomic study and compatibility of this plant with zinc element.

A significant positive correlation between plant weight, leaf width, leaf area and leaf weight indicating that the leaf is more effective than peduncle and bulb in the total weight of the plant, and in producing plants with more biomass weight, larger bulb and longer peduncle, plants that have more leaves are better to be selected. The absence of significant differences among many traits in the plant indicates good adaptability of this plant in different conditions and due to the high differences in some elements of soil and the absence of significant differences among different habitats indicates the lower fertilizer requirement of the plant. Therefore, it seems to be a good option for breeding and production.

Table 2- Variance analysis of *Leucojum aestivum* traits in different habitats

S.O.V	Flower stem lenght	Flower diameter	Leaf number	leaf lenght	leaf width	Leaf surface	large leaf weight	Small leaf weight	Total leaf weight	Total flower weight	main flower weight	Flower number
region	83.9*	8.17ns	3.7ns	125.2**	25.74**	60905**	0.06**	0.035ns	3.58**	0.0003ns	0.0001ns	0.44ns
Error	14.5	1.59	1.12	6.7	0.38	4474	0.0013	0.008	0.21	0.00038	0.00008	0.88
CV%	5.9	6.3	18.4	4.3	4.2	23	6.76	27.59	18.49	19.83	29.2	27.3

ns, no significant; *, significant at P≤0.05; **, significant at P≤0.01.

Continue Table2- Variance analysis of *Leucojum aestivum* traits in different habitats

S.O.V	Bulb weight	Flower stem weight	Plant weight	nitrogen percent	phosphorus percent	Potassium percent	Calcium percent	Magnesium percent	Iron	Manganese	Zinc	Cupper
region	100.5**	44.16*	342.2**	0.05ns	0.0087ns	0.45**	0.001ns	0.0044*	25.14ns	173.65**	488.89ns	955.1*
Error	2.06	6.07	11.51	0.03	0.007	0.23	0.003	0.0007	19.7	14.69	595.9	178.2
CV%	9.98	24.2	12.5	7.5	21.4	8.9	19.3	13.7	22.4	15.5	22.5	21

ns, no significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 3- Mean comparison of *Leucojum aestivum* traits in different habitat.

Region	Flower stem long	Flower diameter	Leaf number	leaf long	leaf width	Leaf surface	large leaf weight	Small leaf weight	Total leaf weight	Total flower weight	main flower weight	Flower number
	(cm)	(cm)	n/plant)	(cm)	(cm)	cm^2/plant)	(g)	(g)	g/plant)	g/plant)	(g)	n/plant)
Tonekabon	62.2ab	18.38b	4.9a	55.7b	12.3c	176.45b	0.36c	0.21a	1.37c	0.08a	0.024a	3.7a
Langerood	59.7b	21.68a	7a	56.3b	18a	449.87a	0.63a	0.39a	3.56a	0.1a	0.032a	3.6a
Lahijan	69.94a	19.91ab	5.3a	66.7a	13.9b	243.6b	0.55b	0.4a	2.5b	0.1a	0.036a	3a

Means in each column followed by the same letter are not significantly different (P≤0.05).

Continue Table 3- Mean comparison of *Leucojum aestivum* traits in different habitat.

region	Bulb weight	Flower stem weight	Plant weight	nitrogen percent	phosphorus percent	Potassium percent	Calcium percent	Magnesium percent	Iron concentration amount	Manganese concentration amount	Zinc concentration amount	Cupper concentration amount
	(g)	(g)	(g)	(%)	(%)	(%)	(%)	(%)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Tonekabon	9.6c	6.7b	17.85c	2.3a	0.46a	2.2a	0.29a	0.24a	413.2a	12.57b	122.8a	73.3a
Langerood	20.85a	14.3a	38.8a	2.56a	0.36a	1.5b	0.27a	0.18b	537.7a	27.7a	104.3a	74.53a
Lahijan	12.6b	9.4ab	24.7b	2.5a	0.38a	1.46b	0.28a	0.16b	281.1a	18.7b	98.4a	42.95b

Means in each column followed by the same letter are not significantly different (P≤0.05).

Table 4- The correlation between traits

Cupper																
Zinc																
Manganese																
Iron																
Magnesium percent																
Calcium percent																
Potassium percent																
phosphorus percent																
nitrogen percent																1
Plant weight															1	0.69*
Flower stem weight														1	0.95**	0.81
Bulb weight													1	0.90**	0.98**	0.6
Flower number												1	0.21	0.24	0.22	0.17
Main flower weight											1	-0.54	0.05	0.11	0.11	-0.005
Total flower weight										1	0.46	0.45	0.39	0.46	0.46	0.22
Total leaf weight									1	0.57	0.35	0.07	0.80**	0.63	0.79*	0.39
Small leaf weight								1	0.58	0.17	0.43	-0.45	0.49	0.54	0.54	0.55
Large leaf weight							1	0.73*	0.92**	0.56	0.54	-0.1	0.80**	0.74*	0.83**	0.55
Leaf surface						1	0.76*	0.29	0.90**	0.49	0.03	0.35	0.89**	0.66*	0.84**	0.33
leaf width					1	0.95**	0.84**	0.44	0.91**	0.4	0.18	0.11	0.93**	0.72*	0.89**	0.38
leaf long				1	-0.16	-0.08	0.21	0.4	0.12	0.14	0.17	-0.09	-0.13	-0.04	-0.07	0.21
Leaf number			1	-0.09	0.82**	0.91**	0.61	0.05	0.83**	0.52	0.07	0.39	0.65	0.37	0.58	0.1
Flower diameter		1	0.72*	0.04	0.81**	0.76*	0.81**	0.65	0.92**	0.39	0.32	-0.1	0.67*	0.46	0.64	0.26
Flower stem long	1	-0.27	-0.25	0.85**	-0.4	-0.32	-0.09	0.1	-0.16	0.18	0.02	0.15	0.3	-0.1	-0.23	0.22
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

17	18	19	20	21	22	23	24
-0.06	-0.13	0.04	-0.2	-0.47	-0.2	-0.14	-0.56
-0.21	-0.51	-0.29	-0.5	-0.09	0.61	-0.04	0.14
0.18	-0.41	-0.67	-0.62	-0.3	0.35	0.08	0.009
-0.17	-0.47	0.04	-0.55	-0.53	-0.03	-0.36	-0.76
-0.29	-0.61	-0.23	-0.55	0.18	0.79**	-0.18	0.15
-0.12	-0.55	-0.37	-0.6	0.02	0.68**	-0.17	0.08
-0.39	-0.82	-0.2	-0.69	0.02	0.81**	-0.43	-0.12
-0.69	-0.6	0.35	-0.32	0.21	0.72*	-0.42	-0.02
-0.2	-0.68	-0.38	-0.69	-0.12	0.69*	-0.2	-0.02
0.3	-0.23	-0.56	-0.34	-0.28	0.27	-0.18	0.007
0.004	-0.41	-0.25	-0.31	-0.19	0.17	-0.07	-0.18
0.36	0.21	-0.36	-0.001	-0.11	-0.04	-0.05	0.12
-0.48	-0.59	0.001	-0.43	0.4	0.92**	-0.35	0.21
-0.59	-0.53	0.15	-0.27	0.51	0.92**	-0.49	0.22
-0.51	-0.61	0.02	-0.41	0.41	0.94**	-0.41	0.2
-0.7	-0.56	0.23	-0.29	0.34	0.70*	-0.62	-0.05
1	0.48	-0.71	0.09	-0.6	-0.69	0.34	-0.05
	1	0.1	0.88**	0.11	-0.63	0.59	0.57
		1	0.46	0.72*	0.27	-0.16	0.24
			1	0.57	-0.32	0.43	0.71*
				1	0.55	-0.15	0.62
					1	-0.51	0.2
						1	0.34
							1

ns, no significant; *, significant at P≤0.05; **, significant at P≤0.01.

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