Investigation of the effect of ecological factors on phytochemical, anatomical and physiological characteristics of the medicinal plant of Vashagh (*Dorema ammoniacum*)

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Abstract

The genus Dorema from the family Apiaceae has 7 species, 2 of which are endemic to Iran, *Dorema ammoniacum* and *Dorema aucheri*. These two species have used by the local population, which are known as Vashagh and Bilhar respectively in Iranian traditional medicine. *D. ammoniacum* has a world-famous gum called Ammoniacum which is very valuable and important in pharmaceutical medicine. This species has grown well in areas with harsh environmental conditions (warm and dry) and inappropriate soils such as Semnan gypsum soils. Many studies have been done on the medicinal properties and active ingredients of gum and its antibacterial, antifungal and anticancer properties that has been used in traditional medicinal to treat neurological and epileptic diseases. Ecological and botanical studies of this species were conducted for the first time in 22,000 hectares of gypsum areas in the west of Semnan. Soil and species analyzes were performed in this paper. Potassium, magnesium and calcium are the highest in *D. ammoniacum.* Relationship between environmental factors and distribution of this species was performed by correlation analysis (RDA). Lime (TNV) and calcium and elevation have a positive relationship with species distribution, but sodium, magnesium and potassium have a negative effect on species distribution. The special relationship between the chemical Components of this species and its ecological conditions was identified and introduced in this study.

Keywords: Ammoniacum, Environment, Photochemistry Components, Semnan

INTRODUCTION

Dorema genus from Apiaceae family contains flat fruits; the yellow perianth is introduced with 7 species in flora of Iran. Species of *D. ammoniacum*, D. aucheri are endemic to Iran, and D. hyrcanum, and *D. aureum* species are endemic to Iran, Afghanistan, and Pakistan. D. ammoniacum is a perennial monocarpic plant that grows up to 2.5 meters height in arid and semi-arid regions of center of Iran [1]. *D. ammoniacum* is a critically endangered species that grows in spring and early summer and has white latex. *D. ammoniacum* is an important endemic medicinal species that grows in most arid and semi-arid regions of Iran such as Isfahan, 88 km southeast of Shahreza at 2150 m height, 35 km south of Shahreza at 200 m height, south of Fars province: Yazd, Shiraz, Sistan, 22 km north of Zahedan, at 1500 m height, Neyshabur and Semnan province: 2-7 km north of Sorkheh at 1300-11400 height, 31 km west of Semnan in gypsy areas at 1200 to 1350 m height and Shahroud, and is known as the Persian names of kandal, Vasha or Koma [2]. This plant is known as medicinal name of dorema ammoniacum, Vasha, and Koma in Iranian traditional medicine. A medicinal gum called Ammaniacum is secreted from the ducts in the stem, root, and peduncle of this plant. This medicinal gum is mentioned in the sources of traditional Greek and Iranian medicine [3, 4]. Ammoniacum is a traditional medicinal plant described in the 10th century by "Abu Mansur Moaffag Heravi" and "Isaac Yahudous" [5, 6]. The resin secreted from the stem prevents insect attack. It is still used as an anti-allergenic English medicinal herb. It is also used for chronic bronchitis and persistent cough [7]. In the past, many studies have been done on the pharmacological properties of *D. ammoniacum*. The antimicrobial activity of dichloromethane-methanol of this species has also been investigated and effective materials of aerial parts of *D. aucheri* and *D. ammoniacum* have been mentioned in various literatures [8, 9]. In Iranian traditional medicine, Ammoniacum

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gum has been used to treat spasms, gastric disorders, intestinal parasitic infections, and skin inflammations and as an analgesic, stimulant, and laxative agent. It has also been traditionally used in Western and Indian medicines as antispasmodics, diaphoretic, and emmenagogue as well as for the treatment of cataracts, asthma, chronic bronchitis, and persistent cough [10, 11]. The antimicrobial and antifungal effects of Amaniacum gum have also been demonstrated [12, 13]. Salicylic acid, ammoresinol, dshamirone, and some sesquiterpene Chromandiones (e.g. ammodoremin and doremin A) have also been isolated and identified from Persian Ammoniacum [14]. There are also some reports about the essential oils obtained from *D. ammoniacum*. It seems that the fruits oil of this species has potent cytotoxic activity on SW-480 and MCF-7 cells, two human cancerous cell lines, and high antimicrobial impacts against Bacillus subtilis and Staphylococcus epidermidis. It has been reported that this plant has 40% ocimenone stereoisomers and 9.9% β-cyclocitral, and important compounds. Previous studies on effective materials of leaf of this plant showed that they are rich in sesquiterpene, which included high levels of α-gurjunene (49.5%) and β-gurjunene (19.0%) [15]. this plant has been used in traditional medicinal to treat neurological and epileptic diseases. Research has also been conducted on the antibacterial and vasodilator properties of this plant [13, 16]. In addition, its extract has also been used. Anticonvulsant is a drug in Greek medicine. The use of gum of this plant in the treatment of neurological diseases, convulsion, and epilepsy has shown positive effects in previous research [17, 18]. Despite extensive reports on the pharmacological properties of this species, there are no reports on the ecological and botanical properties of this species. The present study, in addition to studying the morphological and ecological characteristics of the plant, seeks to investigate the relationship between the botanical and pharmacological characteristics of this species as an endemic species of Iran. In this regard, *D. ammoniacum* species from Semnan that grows in gypsy soils with special ecological conditions is investigated.

Materials and Methods

D. ammoniacum is well distributed in the western part of Semnan and Shahroud in Semnan province. Distribution of this species is especially significant in the gypsy areas of Semnan. The gypsy areas studied in this article cover an area of 22,000 hectares in the western and northwestern districts of Semnan, which includes the villages of Momenabad, Sorkheh, Lasjerd (west), and Aftar village (northwest) (Figure 1).

Figure 1. The white points on the map show gypsum mines

The study area has arid and desert climates. The region's temperature is reported to range from -5° C in winter to 50° C in mid-summer, with an annual rainfall of 192 mm (Islamic Republic of Iran Meteorological Organization, IRIMO 2016). The living conditions of the plants and animals in this area are extremely difficult. The dominant vegetation in this area is low- density and the soil is rich in salts and minerals. In fact, the high salts in the soil and the harsh environmental conditions have brought valuable, consistent, and native plant species to the area that endure harsh conditions. Among these herbs is a traditional native and medicinal species of Iran which is abundant in this region.

For further study of the area, 40 stations and 120 plots (125 m^2) were installed in the area and the distance of plots was 500 meters. Areas of *D. ammoniacum* species were identified. All area information such as latitude, longitude, elevation and slope direction, vegetation including *D. ammoniacum* abundance and other associated species, height, vegetative shape, canopy, flowering time, fruiting time of each plot were collected. The soils of all plots, and especially the soils where *D. ammoniacum* grows, was collected up to 50 cm depth for physical and chemical analysis, including organic carbon analysis, $CaCO_3$, pH, EC, and a variety of soil elements such as calcium, magnesium, sodium, potassium, phosphorus, and nitrogen and then soil texture was determined. Soil analysis was carried out at the Soil Laboratory of Semnan Natural Resources Administration. The minerals of calcium, manganese, zinc, iron, potassium, copper, magnesium and sodium of *D. ammoniacum* were also analyzed in Razi Applied Sciences Foundation Laboratory. The plants were identified in the central herbarium of Semnan Farzanegan University (FGU) by the identification keys of flora Iranica (Rechinger, 1963-2015). The height of study area is between 1000 and 2200 meters above sea level. In addition, photographs of leaf surface cracks of this species were taken using Scanning electron microscope (SEM). RDA (Redundancy analysis) Canoco 4.5 softwares were used to study the ecological relationships, soil and topographic factors of these species. For Anatomical investigations of *D. ammoniacum* transverse sections from *D. ammoniacum* fixed in a 1:1 ratio in alcohol and glycerol. Photographs were taken with the Leitz light microscope (Wetzlar, Nikon camera model Coolpix). Electron microscopy images were taken from the leaf surface of the species.

Results

Geographical coordinates of species

Starting growth of *D. ammoniacum* species in the studied plots was in the late first half of February, at which time a small number of thallus emerged from the soil. This happened earlier in the lower elevations near Sorkheh and later in the higher elevations near the Aftar. D. Ammoniacum together with gypsophila species has formed a special community in this area. Flowering stems and clusters emerge from April to mid-May. Seed formation and ripening occur in June to mid-July. Seeds fall down in mid-July and stem wilt and breaks in August. The seed sleeping period is from September to February. This species creates a beautiful landscape among the low-density vegetation and the herbaceous and cushion-like species, due to its broad and widespread leaves at the vegetative stage and its high height that sometimes reaches more than 2 meters (Figure 2).

Figure 2. D. ammoniacum at flowering stage in Semnan

D. ammoniacum was found in 48% of the sampled plots (at 17 of the 40 sampling stations). This species often grows in gypsy soils at height of 1250 to 2000 m above sea level. The number of species in the plots sometimes reaches 100 (Table 1, Fig. 3).

Table 1. Geographical coordinates of D. ammoniacum in the study area

Stations	Abundance of D. ammoniaceum	slope	Elevation(m)	E	N
1	100	0	1567	35 32.688	53 12.514
2	75	SW	1727	35 34.763	53 7.595
3	20	NE	1738	35 34.707	53 10.565
4	17	0	1796	35 35.869	53 8.504
5	10	E	1776	35 33.778	53 7.865
6	5	0	1294	35 33.638	53 15.409
7	4	S	1608	35 28.758	53 5.209
8	4	N	1969	35 35.693	53 6.218
9	3	0	1423	35 32.088	53 12.739
10	3	N	1663	35 32.575	53 7.8
11	2	0	1462	35 29.131	53 6.876
12	2	0	1492	35 29.413	53 5.269
13	2	SE	1555	35 30.32	53 8.86
14	2	SE	1761	35 31.905	53 9.652
15	2	0	1724	35 34.753	53 10.792
16	1	N	1423	35 31.6	53 12.625
17	1	E	1361	35 31.955	53 13.406

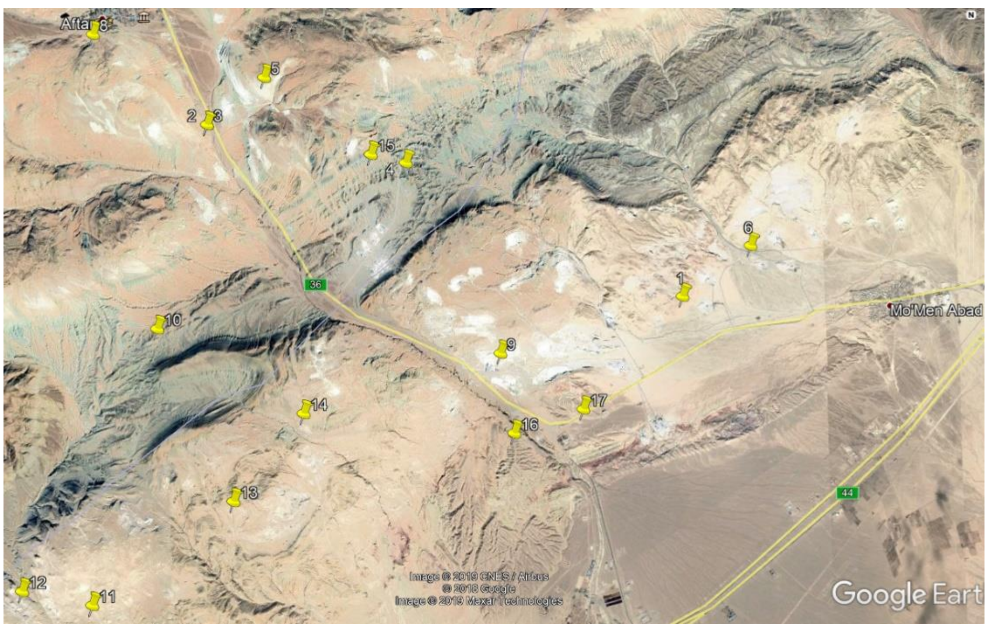

Figure 3. Stations where *D. ammoniacum* species were observed

Soil analysis

The soil of the plots in which *D. ammoniacum* grows was collected from a depth of 50 cm and analyzed. Types of soil analysis included soil texture, pH, EC (salinity), TNV (lime), carbon oxide, nitrogen, phosphorus, potassium, calcium, magnesium, and sodium. Soils of gypsy areas were often flocculated due to high gypsum content. In this region, the average sand content was 74, but the content sand and clay cannot be analyzed due to soil flocculation. The average of soil pH, EC (salinity), and TNV (lime) were 7.5, 2.45 (d.s/m), and 6.5%, respectively. Carbon dioxide, which actually indicates the exchange of carbon ions in the soil, and the amount of soil phosphorus in this region were on average 0.15 and 0.007%, respectively. Soil phosphorus content was also very low and in most plots was zero. However, the soil potassium (82.7 mg/kg), calcium (28.5 Meq/l), and magnesium (12.1 Meq/l) content were significantly high. But the soil sodium content was low (2.8 Meq/l) (Table 2).

Table 2. Soil analysis in the studied plots

Station	Sand %	EC (d.s/m)	PH	Na (Meq/l)	Mg (Meq/l)	Ca (Meq/l)	K (mg/kg)	P (mg/kg)	N %	OC %	TNV %
1	76	2.756	7.50	2.6	8.8	29.6	80	0	0.009	0.12	**18**
2	79	2.309	7.60	1.08	4	31.6	50	0.4	0.003	0.04	**6**
3	45	2.585	7.80	3	21.6	16.8	150	2	0.01	0.14	**13**
4	77	2.373	7.51	1.60	4.8	30	80	3	0.006	0.08	**5**
5	83	2.171	7.68	1.13	23.2	12	50	2	0	0	**1**
6	86	3.952	7.58	28	18	25.6	150	0.8	0.02	0.26	**8**
7	86	2.457	7.80	7	7.2	28.4	50	0	0.008	0.11	**3**
8	63	2.520	7.42	2.82	4.4	33.2	130	3	0.01	0.20	**5**
9	88	2.400	7.40	6	10	25.2	50	0	0.008	0.11	**6**
10	63	2.382	7.75	1.3	11.6	25.6	90	0	0.009	0.12	**2**
11	82	2.253	7.55	7	7.6	26.4	50	0	0.16	0.22	**4**
12	88	3.290	7.58	3	8.8	38.4	80	0.8	0.01	0.14	**7**
13	82	2.912	7.70	1.73	12.8	26	110	0.6	0.01	0.16	**11**
14	69	2.409	7.81	1.3	10.4	27.2	50	2	0.01	0.14	**4**
15	65	2.429	7.78	1.4	16.8	20	70	1	0.009	0.12	**1**
16	80	2.317	7.48	7	3.2	30	60	2	0.005	0.07	**5**
17	88	2.400	7.40	6	10	25.2	50	0	0.008	0.11	**6**

Analysis of Minerals of D. *ammoniacum* species

Analysis of macro and micro elements in *D. ammoniacum* species was also studied in addition to soil elements. Analysis of micro elements such as iron, manganese, zinc, and copper showed that among the micro elements only the iron element had a significant increase compared to the other elements. Among the studied elements, potassium (21,000), magnesium (12,000), and calcium (4000) were significantly found in this plant. Sodium element was low in this plant (Table 3).

Table 3. Analysis of macro and micro elements of *D. ammoniacum*

Elements in plant	mg/kg
Mg	**12000**
K	**21000**
Ca	**4000**
Fe	**13**
Mn	**<1**
Zn	**<1**
Cu	**<1**
Na	**<1**

Mean ecological factors including height of 1608.2 m above sea level, the calcium content of 26.7 mg/kg, magnesium content of 10.8 Meq/l, calcium carbonate or lime (TNV) 8.1%, potassium content of 79.3 mg/kg, and sodium content of 4.8 Meq/l were analyzed (Table 4). RDA analysis with Canoco 4.5 for windows showed that some factors had a positive effect and some factors had a negative effect on the distribution of *D. ammoniacum* (Figure 4). Correlation of environmental factors including calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), calcium carbonate (TNV), and elevation with species distribution is positive and sometimes negative. Calcium carbonate, elevation, and amount of soil calcium have the same direction and positive effect on species distribution while soil salinity factors such as sodium, magnesium and potassium are negatively correlated with species distribution (Table 5). Eigenvalues at the first and second axes were 0.7 and 0.3 and the correlation of species and environmental factors of the first two axes of RDA analysis of *D. ammoniacum* species was 0.8, the sum of Eigenvalues was 1, and sum of all canonical eigenvalues was 0.076 (Table 6).

Table 4. Mean of ecological factors and soil type of studied plots

Name	(weighted) mean	Stand. dev.	inflation factor
Elevation (m)	1608.2	177.0	3.2
Ca (mg/kg)	26.7	6.1	4.9
Mg (Meq/l)	10.8	5.8	5.6
TNV(lime) %	8.1	4.3	1.8
K (mg/kg)	79.3	34.3	3.6
Na (Meq/l)	4.8	6.2	3.3

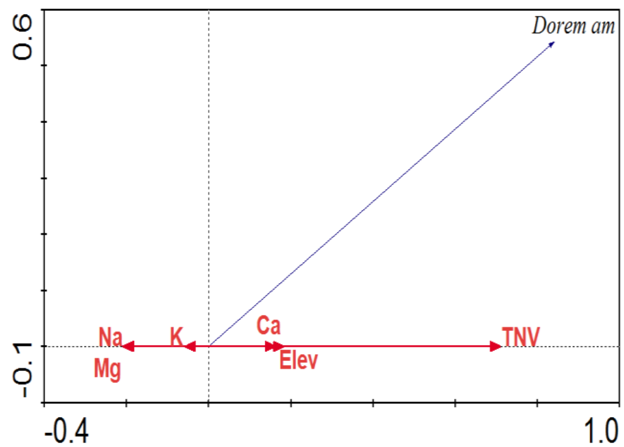

Fig. 4. RDA analysis with Canoco 4.5 for windows shows 6 effective environmental factors associated with the distribution of *D. ammoniacum* species including calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), calcium carbonate (TNV), and elevation. Dorem am: *Dorema ammoniacum*

Table 5. Correlation matrix of *D. ammoniacum* species in the first and second axis of RDA analysis

Environment variable	Ax1	Ax2
Elevation	0.09	0.3
TNV	0.1	0.5
Ca	0.08	0.3
Mg	-0.1	-0.4
K	-0.05	-0.2
Na	-0.07	-0.3

Table 6. Eigenvalues and species correlation and environmental factors of the first two axes of RDA analysis of *D. ammoniacum*

Axes	1	2
Eigenvalues	0.7	0.3
Species-environment correlations	0.8	0
Cumulative percentage variance of species data	70.6	100
Cumulative percentage variance of species-environment relation	100	0

Evaluation of Anatomical and Micromorphological Characteristics of Leaves of D. ammoniacum

Anatomical slices of leaves of *D. ammoniacum* showed black circles or secretory canal on their leaves (Fig. 5). Micromorphological images of leaf surfaces showed that there were many apertures on the leaf surface and their distance from each other was low (50 µm). The interesting thing about these images is the characteristic of *D. ammoniacum* hairs that appear in broad and twisted shapes and their number is very dense in some places. Gypsum particles, which indicate contamination of the area by the presence of gypsum suspended particles in the air and on the leaf surface, are clearly visible in the images (Fig. 6).

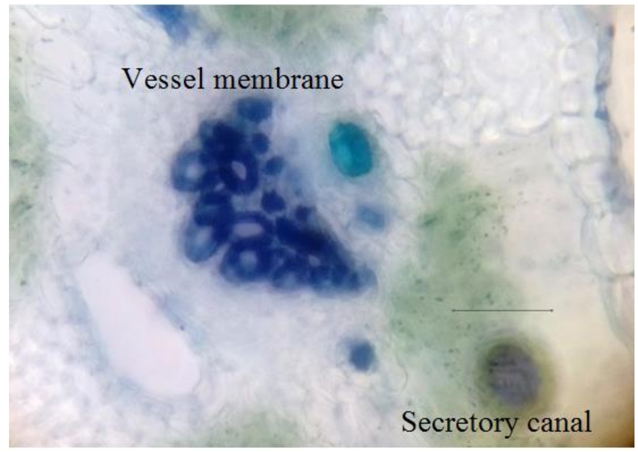

Figure 5. Black circles indicate the presence of gum ducts in the leaves of this species.

Scale bar = 100 µm

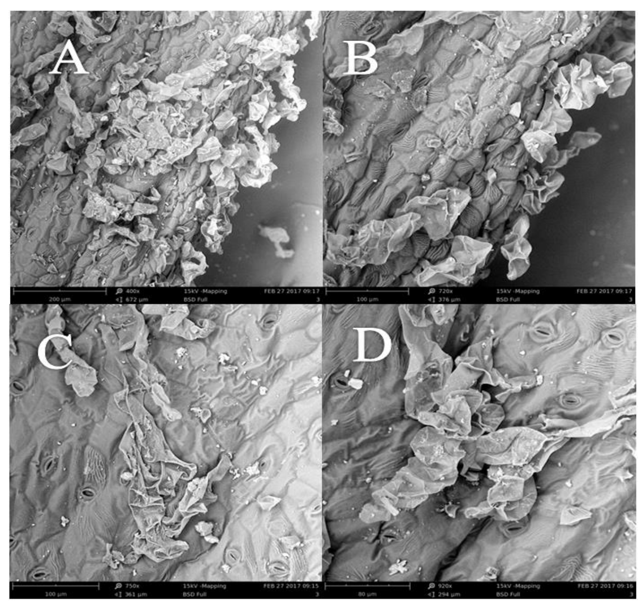

Figure 6. Scanning electron micrograph; A, B, C, D: Leaf surface of *D. ammoniacum*

Discussion

The genus Dorema with endemic species of Iran, *D. ammoniacum*, *D. aucheri*, has been very valuable in traditional medicine. There have been numerous studies to date on the pharmacological, antibacterial, and antifungal properties and for treatment of neurological diseases [10-14]. These two species are widely distributed in the Iranian regions. For example, *D. aucheri* have a relatively wide distribution in the Zagros Mountains. It is also present in areas of central and southeast, Iran-Turan area. Investigations of *D. aucheri* indicate that *D. aucheri* grows in forest-mountains, pasture, highland terrains consisting of calcareous rocks. The highest species density was in the vegetative range of 2300 to 3000 m above sea level [8, 19]. *D. ammoniacum* has been reported in areas of Shahroud and Semnan, and especially in gypsy soils of Semnan [1, 2]. The study of 40 stations in 22,000 hectares west of Semnan and around Sorkheh showed that this species grows at height of 1200 to 2000 meters. Their density is higher in the southern and eastern slopes. Phenology of this species, like other gypsophila species, covers a wide range of gypsum areas with its broad leaves in early spring, then flowering in May. Plant height at flowering stage sometimes reaches more than 2 meters, as previously reported [1]. In late spring and early summer, it produces fruit and seeds and after a month the plant is completely dried and its dried stems remains in different parts of the gypsy area in the hot and sunny region of Semnan in the summer. It often grows along rivers and slopes and is pollinated by bees. Therefore, the plant does not grow in autumn and the seeds are scattered after the fall down by the wind, animals, etc., and begin to grow again after a period of cold and sleep. However, the plant grows through the roots of previous years. Usually, during the rainy season the plant performs its phonology steps earlier. That is, it blooms sooner and the seeds ripen faster and fall. The results showed that this species has a broad root system in both horizontal and vertical directions. So that, the radius of horizontal roots exceeds 160 cm and vertical roots 300 cm, and this feature preserves soil and optimizes moisture utilization by the plant. Therefore, the main role of high canopy volume is to protect habitat soil against heavy autumn rains. This species is a haven for many herbaceous species, insects and other living creatures such as birds, insects, etc. due to their considerable extent and physical volume that the plant is used by animals and humans. Unfortunately, due to the presence of many gypsum mines and digging a mountain, as well as biodegradation and various types of environmental pollution have resulted in serious damage to the *D. ammoniacum* substrate. In addition, grazing animals that feed on the plant have also caused damage to the distribution of *D. ammoniacum* Many animals and insects, including Blattodea, rats, insects, butterflies, flies, birds such as partridge and sparrows, termites, snakes, lizards, rabbits, etc., coexist with this plant, sometimes harming the plant, but some also enrich the physical condition of the soil and move the seeds of the plant elsewhere. *D. ammoniacum* is valuable rangeland, medicinal and edible species that not only plays an important role in animal nutrition but also plays an important role in protecting the soil and the environment, enriching nature and possibly treating certain diseases. *D. ammoniacum*, because of its high reproductive potential and regeneration, can be used as an important species in the regeneration of mountain rangelands in areas with an average annual rainfall of at least 450 mm. *D. ammoniacum* along with other gypsum species such as *Astragalus fridae, Moltkia gypsaceae, Ajuga chamaecistus, Euphorbia bungei, Euphorbia gypsicola, Dendrostellera lessertii, Centaurea lachnopus* and *Dorema ammoniacum*, *Astragalus semnanensis, Acantholimon cymosum, Gypsophila mucronifolia, Jurinea radians* and *Amygdalus lycioides* Create beautiful communities of vegetation in the bare deserts of Semnan. In this study, physical and chemical analysis of the soil, including analysis of organic carbon, calcium carbonate (TNV), pH, EC, and a types of soil elements such as calcium, magnesium, sodium, potassium, phosphorus, and nitrogen was first performed in areas where *D. ammoniacum* grows. The calcium, magnesium, potassium, sodium and calcium carbonate (TNV) content of the soil is significant. RDA analysis by Canoco software showed correlations between environmental factors in distribution of *D. ammoniacum* species (Fig. 4). Calcium carbonate (TNV) had the highest positive effect on distribution of *D. ammoniacum* species, followed by elevation and soil calcium content (Fig. 4). In contrast, magnesium and sodium had the highest negative effect on species distribution, followed by soil potassium (Fig. 4). The correlation matrix of *D. ammoniacum* from RDA analysis also expresses the calcium carbonate (TNV) with the highest impact (0.5) (Table 5). Minerals analysis of *D. ammoniacum* showed that potassium, magnesium and calcium are the highest amounts in the plant, respectively, although potassium and magnesium had a negative effect on the distribution of this species based on correlation analysis. Among the micro-iron, manganese, zinc and copper elements, the iron content was highest at about 13 mg/kg, but the rest of the elements were less than one mg/kg. In all leaf anatomy slices, such black circles were observed, indicating secretory canals (Fig. 5). Low stomata distance and high number of stomata at leaf surface indicate that photosynthetic efficiency is increased in this plant. The existences of wide and diverse hairs in this species have provided conditions for living in warm and dry conditions of Semnan. The amount of calcium in the studied soils is about 30 Meq/l on average and the amount of calcium in *D. ammoniacum* is 4000 mg/kg. In fact *D. ammoniacum* is extremey halophobe and it is calciphyte or calciphile and absorbs calcium which, according to the correlation chart shown by RDA analysis, lime ($CaCO_3$) has the highest positive effect on *D. ammoniacum* growth, but according to this chart, magnesium and potassium content in the soils of this region indicates the presence of salts in these soils. But according to correlation analysis of RDA diagrams, magnesium, sodium and potassium factors play the most negative effect on species distribution, respectively. Calcium is an important factor in plant salt tolerance, especially important for sodium and potassium homeostasis. Calcium is considered to be an important nutrient in plants exposed to NaCl due to its role in reducing sodium uptake and increasing K^+ and Ca^{2+} uptake [20, 21]. High concentrations of Ca^{2+} can decrease the permeability of the plasma membrane to Na + and alter the cell wall properties leading to decreased Na + accumulation by inactivation [22]. A special relationship was observed between the elements in the soil and plant of this species with the composition of the active ingredients of this species. Ammoniacum gum active ingredients of the study area by Yousefzadi *et al*. (2011), which showed that some of the active ingredients such as sesquiterpenes in this species are much higher than other compounds in Ammoniacum gum. Mg ion as a cofactor is usually required to form the structure of sesquiterpenes [23]. However, the Mg ion is generally accepted as a physiological cofactor. Other metals, such as K, have been shown to have a profound effect on the catalytic performance of some sesquiterpenes synthases [24-26]. This paper concludes that the highest elements present in *D. ammoniacum* are magnesium and potassium at 12,000 and 21,000, respectively, which are two of the elements required for the formation of sesquiterpenes synthases. In fact, this mechanism, the accumulation of ions in the plant gum, in a way, helps to liberate the plant from the abundance of elements in its soil, making the plant adaptable to the harsh ecological and demanding conditions such as Semnan gypsum soils.

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