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BIOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF A MONOTYPIC RELICT ENDEMIC SPECIES: *DORYSTOECHAS HASTATA* (LAMIACEAE)

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Abstract

In this study, morphological and ecological properties of Dorystoechas hastata Boiss. & Heldr. ex Bentham which is a species belonging to Lamiaceae family was investigated. D. hastata has 40-100 cm lenght; with pale roots; deeply branched, woody shrub and globular stem; aromatic; leaves are lanceolate-hastate, 2.2-3.5x5.1-8.7 cm, with dense hairs; infloresence is a spica; kalix lenght is 3.48-4.23 mm in flower, 4.6-7.6 mm in fruit; corolla white and 4.3-6.9 mm; pollens which are isopolar, tricolporate and 60x100 µ; fruit is a light brown and bright nutlet. D. hastata distributes in areas which have climate like Mediterranean type, above calcerous main rock, dominant soil characteristic is arenaceous-silty arenaceous, among elements which have xerophyte macchie characteristics. Seeds of this plant germinates % 1.25 at light and % 1 at dark media. Additionally, statistically significant relationship among plant morphological characteristics and physical and chemical characteristics of the soil were determined.

Key words: Dorystoechas hastata, Ecology, Endemic, Lamiaceae, Monotypic and Relict

INTRODUCTION

Dorystoechas hastata Boiss. and Heldr. Ex Bentham is a monotypic relict endemic species of Turkey. The province of Antalya (Beydağları) which is *Dorystoechas hastata* Boiss. & Heldr. ex Bentham's natural range (Figure 1) is located in Eastern Mediterranean area of Holoarctic Region. In the Mediterranean region, which is a very old settlement, the type of vegetation is dominated by shrubs because of destruction. However, the rate of endemism in the region is close to 50%, especially Tertiary relicts were seen more than young species (Atalay, 1994). Milne and Abbott (2002) pointed out that Anatolia is the area which tertiary relict floras were formed and possible intercontinental migration routes. Lamiaceae is a cosmopolitan family includes about 200 genera and 3000 species in the world and in Turkey it has 45 genera and more than 546 species (Seçmen et al., 2000). Walker et al. (2004) observed that *Salvia* species were close relatives to *Dorystoechas* by phylogenetic analysis performed on two different region of chloroplast DNA of 19 genera which are belonging to Lamiaceae family. One of the main characteristics of Lamiaceae members is rich chemical content they carry; it's known that *D. hastata* includes 1.8-co-cineol (Başer, 1994), kampherol (Valant-Vetschera et al., 2003), 6-OH-luteolin, 6-methyl ester from leaves (Venturella et al., 1988), as the main component of essential oil: 1.8-cineol, a-pinene and borneol mainly guaiol, camphene, camphor (Başer and Öztürk, 1992), ferrugineol from roots, 6.7-didehydrosempervirol 17-hydroxicriptotansinon, przevacuinon A and criptotansinon 17 betaoic acid

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(Ulubelen et al., 2004). *D. hastata* were found to be very effective antioxidative extracts, like chlorogenic, caffeic, p-coumaric, ferulic and rosmarinic acids as phenolic acids, quercetin, kaempferol and apigenin as flavonoids and carnosic acid and carnosol as diterpenoid antioxidants in the plant (Erkan et al., 2011). Although *D. hastata* used was not used as an ornamental plant, it's already grown in botanical gardens in Europe (Valant-Vetschera et al., 2003). *D. hastata* plant is locally named as "Calba" and is used for the preparation of an aromatic tea (Başer and Öztürk, 1992). Karagözler et al. (2008) studied the proline and antioxidant contents of the leaves and showed that the species may serve as a natural source of proline and antioxidants. Fresh or dried *D. hastata* leaves are used to make an aromatic tea, locally known as chalba tea, with a pungent taste, which is used as a healing beverage against common cold or as a health drink by the local inhabitants (Kan et al, 2015; Karagözler et al., 2008). On a study about acaricidal effects of Lamiaceae essential oils, Koç et al. (2012) found out that the essential oil of *D. hastata* is very toxic by killing 100 % of the larvae of *R. turanicus* at all concentrations.

In this study, it's aimed that to determine the biological and ecological characteristics of *D. hastata* which is the only species is belonging to the genus *Dorystoechas*.

MATERIAL AND METHODS

Specimens collected from research area were prepared as herbarium material and placed in ANES (Anadolu University Faculty of Science Herbarium). Morphological characters were measured by digital caliper under stereomicroscope if they were small structures, and by scale if they were large structures. These characters were determined by taking the average of the results obtained on four separate plant samples collected from different regions, by measuring four time.

To determine the soil characteristics, soil samples have been taken depths of 0-10 cm, 10-20 cm and 20-30 cm of sample plots. Bouyoucos Hydrometer Method was used to determine texture (Bouyoucos 1962). Soil reactions were measured by Beckman pH meter, (±) 0,01 sensitivity, Jackson Method (Jackson, 1962) accordingly. To calculate the amount of moisture in the soil, samples were sieved (2 mm Ø), kept in a drying oven at 105°C overnight (Çepel, 1966). Determination of calcium carbonate in soil samples was made by Volumetric Method (Toker and Schomak, 1963). Determination of the amount of organic matter in soil samples is determined by Wackley-Black's Wet Decomposition Method (Wackley and Black 1934). Determination of nitrogen content of soil samples was carried out by Semi-Micro Kjeldahl method (Jackson, 1962), kjeltec Auto 1030 Analyzer distillation equipment was used. The amount of phosphorus contained in the soil sample which can be used by plants was performed according to the method of Olsen (Aydeniz, 1969), Spectroconic 20 was used as device. Ca, Mg, K and Na analyzes of soil samples were performed according to the method of Ammonium Acetate (Walsh and Beaton, 1973). Determination of the Fe, Cu, Mn and Zn in soil samples is determined by Wackley-Black's Wet Decomposition Method (Wackley ve Black, 1934). Prepared soil extracts were analysed by using Perkin-Elmer 3030 B Atomic Spectrophotometer device, 3 repeatedly, the sensitivity was 0.01 ppm.

Plant sample analyses were made: Nitrogen by Semi-Micro Kjeldhal method (Jackson, 1962); phosphorus by Olsen method (Aydeniz, 1969); calcium, magnesium, potassium by ammonium-acetate method; sodium by sodium acetate method (Jackson, 1962); iron, copper, zinc and manganese by wet decomposition method (Wackley and Black, 1934).

Germination experiments were performed in plant breeding cabin (ML-350 Model Sanyo, Japan). Experiments were carried out in Petri dishes (9 cm Ø) and germination bed formed from filter paper. During the tests temperature was kept at 22°C±1ºC and held white light source (12 h ligth/12 h dark photoperiod) was used. In each experiment, 100 seeds were used for each concentration. Germination tests were performed according to Yucel (2000). Duncan test were performed to data obtained from germination experiments.

RESULTS

It's very hard to compare these results given because *Dorystoechas hastata* is a monotypic endemic plant, there is no other species from the same genus. *D. hastata* plant samples were collected from its natural

distribution areas (Table 1). Localities were located between 30-970 m elevations. It was observed that the most widely distribution is in Termessus National Park, it can be said that in this region taxon is far from andropogenic effect.

Morphological characteristics: *D. hastata* is a perennial, aromatic, densely hairy, and woody rooted, branching from the bottom, evergreen shrub (Fig. 2.a). The substrate is limestone and plants extend out from the rock crevices. It was found that plant height varies 70-140 cm (Table 2). Root is woody, tap, lentgh is 18.9-29.5 cm, thickness is 1.7-2.4 cm. Lateral roots are scarce and do not exceed the lentgh of the main root. In young roots, under the cortical layer it has a reddish color due to chemical contents (Ulubelen et al., 2004). The stem is woody, branching starts from the bottom, a main stem can not be observed. Stems which are grown in open areas has a spheroidal form, however some individuals grown up in areas where light is scarce has a pendant. Cortex on stem has lots of longitudinal hiatuses and can not be easily peel off. The leaves are fragrant, lanceolate, hastate, 2.2-3.5x5.1-8.7 cm, often grayish hairy both sides, wavy, small carved; petiole is covered with similar hair as leaves, 1.9-2.4 cm in length and slightly curved from before bazis.

Spica is 6.8-13 cm length, verticillaster is 10-25 flowered, and peak of the spica has less flower number (Fig. 2.b). Pedicels are 0.6-0.9 mm, it can not be distinguished easily from the calyx. Flowering starts at May, reduced to a minimum at July. Calyx is 3.5-4.2 mm in flower, reaches 4.6-7.6 mm in fruit; narrow angled in spica axis; the outer surface covered with an intensive multi-cellular hairs and a small number of glandular hairs, inner surface has less simple and glandular hairy; upper lip has three indistinct teeth, lower lip has two obvious teeth, those teeth are 1.4-1.8 mm. Corolla is white, 4.3-6.9 mm; petals united, but significantly two-lipped, upper lip split into two parts, the lower lip is longer than the upper lip with three lobs. Fruits are drupes, permanent until the next flowering season. Fruit type is nutlet, light brown and shiny, 0.6-0.9x1.6-2.3 mm.

ECOLOGICAL CHARACTERISTICS

Soil samples: In the research area, bedrock is the limestone and geological structure is the Upper Cretaceous (Duberted et al., 1973). In this study, it was identified that the *D. hastata* prefers limestone rocks which are very rich in lime and located in areas with sandy soil as substrate. The physical properties of the soil in the distribution area of *D. hastata* are given in Table 3. The physical structures of the soil are very similar in locality 2 and 3. From 0-10 and 10-20 cm depth soil examples of locality 2 and 3 were "sand", and samples taken from depth of 20-30 cm were "sand-clayey sand". For locality 1, soil samples taken from all depths were "sand" (Table 3). The chemical structure of soil in the distribution area of *D. hastata* is also given in Table 3.

Nutritional element and heavy metal content of plant samples: The amount of nutrient elements in the leaf, stem and root, collected from three different sample areas, are given in Table 4. The heavy metal content in the leaf, stem and root, collected from three different sample areas, are given in Table 5.

Seed germination characteristics: The highest germination percentage of all series was observed in 0.5% NaCl application by 9.75%, while germination rate was calculated as 11,15 (Figure 3). For 0.5% NaCl application, highest germination percentage was observed in 0.5% KNO3 application as 6%. For this application, germination rate was recorded as 14.8, which is the highest germination rate in the series. In other series (1-2-3% NaCl, 2-3% KNO_3, all applications of HCl and H_2SO_4) no germination was observed.

According to data, 12 h light/12 h dark photoperiod and dark applications have similar results in point of germination percentage, while 1% KNO_3 application also participated in this homogeneous group ($F=13,820$, $df=17, 54$, $P<0.005$). 0.5% KNO_3 application forms a second homogeneous group alone; likewise 0.5% NaCl application. The largest non-homogenous group is the germination tests which no germination was observed. According to the statistical analysis, compared the seed germination rate, 12 h light/12 h dark photoperiod and dark applications have similar results, and 1% KNO_3 application also participated in this homogeneous group ($F=13,105$, $df=17, 54$, $P<0.005$). Another homogeneous group, 0.5% KNO_3 and 0.5% NaCl applications are separated from other applications; test series observed no germination were created a homogeneous group.

DISCUSSION

In this study, monotypic relict endemic *D. hastata* was examined in terms of biological and ecological aspects, which was distributed only the area of Antalya (Turkey) province, limited to Beydağları. Floristic, phytochemical and phylogenetic studies have been conducted about this taxon so far, although there is no individual ecology study about it. This taxon, for the first time, was examined regarding both biological and ecological aspects by this study. In the literature, the morphological characteristics' data related to this species only found in The Flora of Turkey (Davis, 1988). The differences were found between this study and The Flora of Turkey in terms of morphological characteristics and detailed description of the species. In this study, the morphological data was updated, compared with Davis (1988), and missing data has been completed (Table 6).

Data shows that the error in the lower and upper limits of the morphological characteristics specified in Flora of Turkey. Additionally, in this study, root length, root and leaf thickness measurements were also made. As a result of intensive studies it was found that indumentum of *D. hastata* was formed different types of hair. In the literature it was found that secretory organs and soft hair cover (Davis, 1988), in addition, we found out that *D. hastata* has stalked glandular and tooth-like hairs in leaves, and simple, multicellular and stalked glandular hairs in the calyx. Leaves are thick, pilose and often curved, it was concluded that the structure of plants is appropriate for xerophytic the Mediterranean region.

This study is the first report on soil characteristics of *D. hastata*'s distribution area. *D. hastata* is a casmophyte species and soil samples of distribution area were "sand" between in a depth of 0-10 and 10-20 cm, while "sand-clayey sand" in 20-30 cm. Soil samples taken from Beycik locality were "sand" in all depths. In the distribution area, in terms of chemical properties, soil samples were light and medium alkaline, and lime and very chalky class. The heavy metal content of soil and plant samples were lower than the limit value announced by the European Community Council (Winsemius, 1986) and the TMMOB Agricultural Engineers Chamber (Official Gazette, 2003), so we can say that there is no pollution threat.

Kan et al. (2015) reported that the most abundant minerals in *Dorystoechas hastata* were potassium in the branch, phosphorus, magnesium and calcium in the leaf and sodium in the whole aerial parts. In contrast to Kan et al. (2015), we found that the highest amounts of potassium was found in leaves, sodium in stem and the lowest amounts of phosphorus in leaves. Similarly to Kan et al. (2015) we found out that the highest amount of calcium and magnesium were in leaves. This is the first report on nitrogen content of *D. hastata* plant materials. We found out that iron, copper, zinc and manganese have different levels among plant organs, not condense in just one type of plant organ, inspite of Kan et al. (2015) reported that iron, manganese, zinc and copper were in minor amount in the leaf.

In a research about *D. hastata*, the highest germination rate (70%) was found with vitamin-supplemented distilled water containing 1 mg L^{-1} of GA_3, White's medium and MS medium, both with 1 mg L^{-1} of GA_3 added, showed lower germination rates, 45% and 35%, respectively (Erdağ et al., 2010). Erdağ et al. (2010) suggest that gibberellic acid added to the media breaks dormancy and hastens germination of *D. hastata* seeds. In this research, the highest seed germination percentage was observed at 0.5% NaCl applications, but this is not the highest germination rate, it was 0.5% KNO_3 application. Germination rate under light conditions is higher than dark conditions for this species. Germination ability of the *D. hastata* seeds were inhibited in acidic media. In an alkaline environment, the seeds exhibit more rapid germination capability. However, there is a very low value of the seed germination and give negative ideas about the future of populations of this species. Seedlings formed by germinating seeds is very weak, and decay rapidly. In nature, it seems very difficult to generate new individuals through germination. In area research, it was observed that young stems were formed from between root and stem in many individuals. Poor seedling structure, low germination ability and the vegetative reproductive ability of *D. hastata* shows that vegetative reproductive ability of this species is higher than generative ways.

D. hastata's category of danger was identified because of being an endemic and having restricted distribution. Danger category of this species is VU (Vulnerable) (Ekim et al., 2000). In Kemer locality, individuals

were in an open area and faces the grazing danger. In Beycik locaity, it was observed that leaves of this plant were used as herbal tea and the villagers cut this plant if they do not use it. Thermessus National Park, another locality, intensely open to tourism, is reported to visitors it is forbidden to harm the vegetation and the number of individuals is higher compared to other localities. This indicates that the taxa exist in protected areas developed better.

Kan et al. (2015) observed that differences in the composition and yield of the *D. hastata* oils appear to be climatic, seasonal, geographic, soil and other cultivation conditions, as well as the part of plant and the harvest period. All of the oils of *D. hastata* had a strong, refreshing, pleasant aroma which may be an interesting new source of fragrance to the perfumer, however, it has limited distribution, the best solution would be to start agricultural studies for large scale cultivation (Başer and Öztürk, 1992). Due to different classes of phenolic compounds detected, namely phenolic acids, flavonoids and diterpenoids, and the strong antioxidant capacity, *D. hastata* can be consumed as a potential antioxidative edible source (Erkan et al., 2011). *D. hastata* may be a good source of components that would help to increase the overall antioxidant capacity of an organism and protect it against lipid peroxidation, induced by oxidative stress (Karagözler et al., 2008). Kan et al (2015) suggest that *D. hastata* can be used as natural ingredient source in pharmaceutical and food processing including veterinary applications.

Because of its interesting shaped leaves, small white flowers and nice fragrance, this species can be used as an ornamental plant. *D. hastata* is suitable to be preserved and grown as an ornamental plant in botanic gardens. With the medical importance, *D. hastata* can contribute to the economy by cultivation.

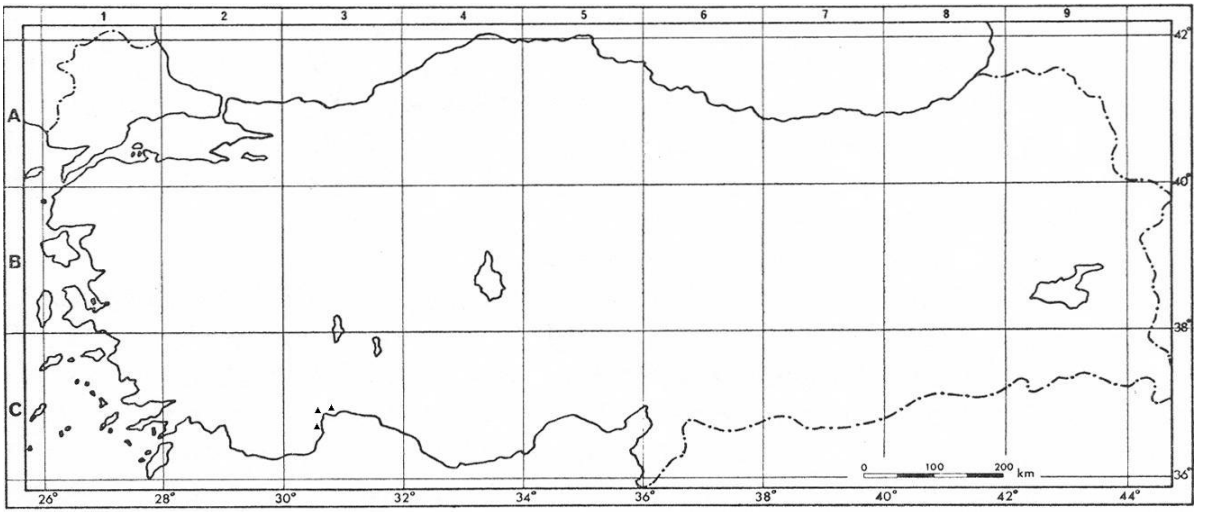

Figure 1: The distribution areas of *D. hastata* ( )

Table 1. Localities forming the research area

Locality No	Locality	Altitude	GPS
1	C3, Antalya, Kemer, Beycik Village, İnderesi	757 m	N 36º 30’ 29.2” E 30º 25’ 38.1”
2	C3; Antalya, Termessus National Park, Toptepe	967 m	N 36º 59’ 07.2” E 30º 27’ 49.1”
3	C3; Antalya, Kemer road, Sarısu	30 m	N 36º 50’ 05.2” E 30º 36’ 04.5”

Figure 2. a.The general appearance of the vegetative stage of *D. hastata* (Termessus), b. Spicas of *D. hastata*

Table 2. Average measures of *D. hastata*'s morphological characteristics

Morphological characteristics (mm)	Locality 1	Locality 2	Locality 3
Root length	224.75	189.00	294.50
Root thickness	16.72	19.39	24.42
Plant length	1431.50	718.75	770.50
Leaf length	87.31	50.77	64.58
Leaf width	34.64	21.54	24.47
Leaf thickness	0.63	1.02	0.87
Petiole length	23.53	18.99	19.82
Spica length	107.75	130.25	68.00
Pedicel length	0.89	0.89	0.57
Calix length at flower	4.23	3.58	3.48
Calix length at fruit	7.64	5.29	4.64
Calix tooth length	1.80	1.43	1.42
Corolla length	6.87	5.46	4.26
Fruit width	0.64	0.99	0.92
Fruit length	1.63	2.32	2.33

Table 3. Physical analysis, pH degrees, $CaCO_3$%, % organic matter, macro elements and heavy metals contents of soil samples

Locality	Depth	% Sand	% Clay	% Silt	Texture	pH	% $CaCO_3$	% Organic matter
1	0-10 cm	94.84	4.1	1.06	Sand	7.9	41.75	2.19
	10-20 cm	91.04	1.4	7.56	Sand	7.85	38.77	2.55
	20-30 cm	93.4	5.76	0.84	Sand	7.8	37.28	3.08
2	0-10 cm	99.4	0.4	0.2	Sand	7.55	12.98	17.64
	10-20 cm	95.4	3.37	1.23	Sand	7.55	12.98	18.49
	20-30 cm	85.4	3.76	10.84	Sand-Clayey sand	7.6	14.47	14.47
3	0-10 cm	93.4	5.26	1.34	Sand	7.6	18.64	20.35
	10-20 cm	92.1	4.9	3	Sand	7.6	19.38	21.12
	20-30 cm	89.4	9.4	1.2	Sand-Clayey sand	7.7	19.38	19.7

Continued table 3…..

Locality	% N	P (ppm)	Na	K	Ca	Mg	Fe	Cu	Zn	Mn
1	0.2292	89	40	260	4187	167	0.06	0.2	1	2.3
	0.3852	90	40	290	4971	223	0.02	0.2	1.1	3.6
	0.3199	92	40	360	4580	231	0.02	0.1	1	1.4
2	7.5745	83	70	700	7956	263	0.08	0.4	1.2	0.8
	7.1838	83	70	760	7945	247	0.08	0.5	1.4	0.7
	5.8037	81	70	850	7687	220	0.09	0.4	1	0.8
3	3.4179	84	60	500	8314	299	0.11	0.4	1.1	1.6
	6.0525	88	70	580	8251	352	0.14	0.4	1.3	2.3
	5.5679	84	70	580	8122	312	0.12	0.4	1.7	2

Table 4. Nutritional element content of plant samples

Locality	Organ	% N	P (ppm)	Na	K	Ca	Mg
1	leaf	1.3098	60	500	28500	15210	1570
	stem	0.6903	86	600	16000	8120	840
	root	0.7410	84	450	14500	8190	870
2	leaf	1.3443	61	300	22500	19140	1225
	stem	0.9171	84	400	18000	10370	895
	root	0.9640	85	400	18000	13600	1090
3	leaf	1.3417	57	300	25000	18405	1265
	stem	0.8133	82	400	21000	11510	985
	root	1.0883	85	350	7000	18730	780

Table 5. Heavy metal content of plant samples

Locality	Organ	Fe (ppm)	Cu	Zn	Mn
1	leaf	8.02	12.5	31.5	28.5
	stem	4.25	8.5	32.5	14
	root	3.81	8.5	34.5	14.5
2	leaf	4.37	10.5	47.5	45
	stem	7.28	9.5	32.5	28
	root	10.06	7	20	42
3	leaf	4.41	4.5	35	34,5
	stem	5.54	3.5	32.5	22
	root	14.06	10	42	51.5

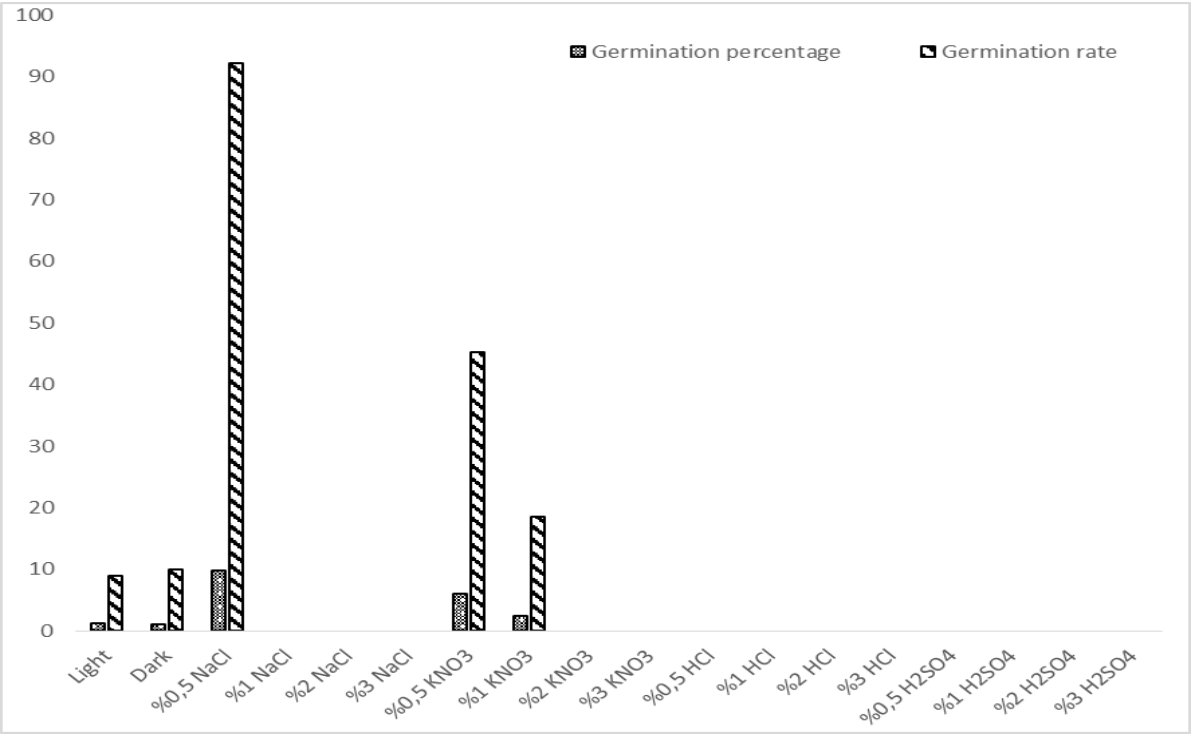

Figure 3. Germination percentage and germination rate of the *D. hastata* seeds

Table 6. The comparison of morphological characters measured with literature data

Morphological characters (mm)		Flora of Turkey (Davis, 1988)	In this research
Root	length	-	189-295
	thickness	-	1.7-2.4
Plant length		400-1000	700-1400
Leaf	width	15-30	51-87
	heigth	28-75	22-35
	thickness	-	0.6-1
Petiole length		Petiole length	18.99-23.53
Spica length		Spica length	68-130
Pedicel length		Pedicel length	0.6-0.9
Calix length	At flower	3-4.5	3.48-4.23
	At fruit	6-10	4.6-7.6
Calix tooth length		1.5	1.4-1.8
Corolla length		4-6	4.3-6.9
Fruit	width	1	0.6-0.9
	heigth	2.5	1.6-2.3
Pollen	width (µ)	-	60
	heigth (µ)	-	100
		-	189-295

CONCLUSION

Dorystoechas hastata Boiss. and Heldr. Ex Bentham are monotypic relict endemic species and distributed around different parts of Turkey. We determined biological and ecological characteristics of *D. hastate* in the course of this study. Revealing seed germination behavior of these species were very important for the future of country population. Conservation and cultivation policies must be adopted for these taxon immediately.

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