

## Conservation Strategy of Critical Endemic *Centaurea hausknetchii* Boiss. (Section: *Cyanoroides*) and Effects of Different Salt, Nitrate and Acid Concentrations on the Germination of Seeds

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*Centaurea hausknetchii* is a local endemic species growing only in the Nemrut Mountain Historical National Park in the South-east Anatolian region of Turkey. It grows in three small areas of this mountain and all these areas are under heavy grazing pressure. The germination behaviour of the seeds was studied and for this purpose seeds were left under two different photoperiods (8 h light-16 h darkness and 16 h light-8 h darkness) and treated with different concentrations of NaCl, KNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and HCl solutions. There was no germination in 3 % NaCl, 1-3 % KNO<sub>3</sub>, 1-3 % H<sub>2</sub>SO<sub>4</sub> and 2-3 % HCl concentrations in each photoperiod. According to the univariate variance analysis of germination test results, germination media with different salts, nitrate and acid concentration (e.g., 0.5 %, 1.0 %, 2.0 % NaCl; 0.5 %, 1.0 %, 2.0 %, 3.0 % KNO<sub>3</sub>; 0.5 % H<sub>2</sub>SO<sub>4</sub>; 0.5 %, 1.0 % HCl) under different photoperiods (8 h light-16 h darkness and 16 h light- 8 h darkness and 24 h light) and control have been investigated. The photoperiod and photoperiod-treatment interaction had a significant impact on the germination percentage and rate ( $p < 0.05$ ). *C. hausknetchii* is in the critically endangered category (CR) in the IUCN 2000 Red Data Book. The habitat characteristics of the distribution sites were thus investigated, vegetative growth behaviour followed and inter-relations between the increase and spread of populations recorded. The factors effecting the productivity were determined. This report will be helpful in the population biology studies and development of alternatives for effective protection programme.

**Key Words:** *Centaurea hausknetchii*, Endemic, Autecology, Conservation.

### INTRODUCTION

Geological, paleogeographic and historical factors have helped to create highly diverse environments. One of the most important criteria used in the identification of high-priority areas for conservation is endemism<sup>1,2</sup>.

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Thus, the insular, mountain or isolated edaphic systems (ultrabasic rocks, gypsum, limestone, *etc.*) generally appear to be major endemic centres. However, in some areas a high number of endemics may not correspond to high species richness<sup>3</sup>. The impact of humans on natural ecosystems has resulted in the formation of a new suite of rare species that were previously more abundant but are now rare because of human disturbances. In many rare species classifications, including the Red Book listings of the IUCN<sup>4</sup>. Rarity is a normal feature of all biological communities with most species assemblages comprising a few abundant species and many with only a few individuals<sup>4</sup>.

These two types of rarity, natural and induced, are not always clearly distinguished<sup>4,5</sup>. As a result, some species that are naturally rare are also ranked as threatened with extinction. The understanding about the difference between natural and induced rarity is important for focusing conservation efforts<sup>5</sup>. Such habitats are of small extent within a region and present particular habitat limitations (*e.g.*, exposure, water shortages, lack of soil). They can also be important refugia for widespread species because climates and habitats often escape disturbances such as fire<sup>6</sup>. Due to this isolation and unique environment, cliffs provide habitat for local endemic and naturally rare plants<sup>6,7</sup>.

Turkey has an extremely rich flora due to its geographical location, ecological properties, paleogeography and vegetation history. Although, Turkey has one fifteenth of total land covered by European countries, it has an overwhelming number of endemic species. The records show that the European countries other than Turkey possess 12000 species of which 2750 are endemic<sup>8</sup>.

Turkey, in particular the south-west and east of the country, is one of the main centres of diversity for the genus *Centaurea* L.<sup>9,10</sup>. The total number of *Centaurea* species known from Turkey has increased to 190<sup>11</sup>. High endemism ratio shows that Turkey is one of the gene centers of this genus.

For the purpose of *in vitro* breeding of *C. hausknetchii*, germination ecophysiology investigations about this species were made. Seed germination and early seedling growth are critical stages for the establishment of plant populations in their natural habitats<sup>12</sup>. The importance of light depends upon the size of the seed, its life form (annual or perennial) and the habitat of the species. Addition of exogenous KNO<sub>3</sub> too influences germination during this step. Recent physiological models have implicated an interaction of light quality, soil nitrate, in light-affected germination<sup>13</sup>. The effects of NaCl, KNO<sub>3</sub> and light in particular and the interactions between these factors in the germination behaviour of *C. hausknetchii* were investigated. Rapid industrialization, population increase and urbanization bring important environmental problems in their wake. With the complexity and extensiveness

of acid rains, increased salinity decreased and alkinitiy are particularly threatening to Turkey soils<sup>14,15</sup>. Due to increasing salinity, 0.6 % of Turkey's land area is no longer used for cultivation<sup>16</sup>. As a result, the risk of further salinification and consequent barrenness in currently fertile areas requires serious and urgent consideration. Salt-tolerance in plants may have a direct relation to growth<sup>14</sup>. It was attempted to identify the germination medium for essential to culture of the species *Centaurea hausknetchii*, in point of landscape ecology and depletion danger. Identifying the germination capacity and speed of the species is important in terms of conservation of biodiversity.

In this study, ecological aspects of the populations of this species which is belonging in to critically endemic category, distributions, treats which are this species was exposed, were particularly hand out. In this study an attempt is made to put forth ecological behaviour of of *Centaurea hausknetchii*, a rare and critically endangered endemic species.

### EXPERIMENTAL

*C. hausknetchii* is distributed only in the Nemrut mountain Historical National Park in the vicinity of Adiyaman in southeastern Anatolian region of Turkey. Nemrut mountain is the first impressive peak rising from a flat plain in North Mesopotamia and stretches to a height of 2150 meters. This species is known only from 3 populations 90 km in the north of Adiyaman, district of Kahta, around of Nemrut Mountain National Park. These populations are located on serpentine and agglomerate habitats close to the roads in tragacanthic steps. The climate of the area shows that annual mean temperature is 11 °C and average precipitation 674-730 mm. 53.8 % (414.0 mm) of the rains fall in winter. Average temperature is below 7 °C between November and March, but mean minimum is -22 °C in December and mean maximum 26.9 °C in June. In summer mean temperature is 29.0 °C and evaporation is 66 % (annual average being 117.9 mm). Soils are water deficient between June and September (215 mm-dry period)<sup>17</sup>. Forest vegetation is very poor. In the study area climate is dry and vegetation is steppe.

Plant specimens belonging to *Centaurea hausknetchii* Boiss. and its associates were collected from three known localities in Adiyaman around Kahta Province. The plant specimens were identified according to Wagenitz *et al.*<sup>9</sup> and they are stored in the Herbarium of Biology Department in the Canakkale Onsekiz Mart University, Turkey. The morphological observations and biometric measurements were made on fresh as well as herbarium specimens. Population density and relations between the species were followed for two years (2002-2004) to record the competition behaviour. Soil samples (0-30 cm deep) were collected from the area where the plant specimens were growing and brought into the laboratory for physical and chemical analysis (Table-1) according to the methods outlined by Ozturk *et al.*<sup>18</sup>.

Germination experiments were done in a growth cabinet (MLR-350 Model Sony, Japan). A constant temperature was used ( $25 \pm 1$  °C) and two photoperiods (8 h light, 16 h dark daily photoperiod, 16 h light, 8 h dark daily photoperiod) were used. In each experimental series 100 dark coloured seeds were utilized per dish and experiments replicated four times ( $4 \times 100$ ). The seeds were left on a filter paper over glass plates in 9 cm petridishes. The 6 series of experiments were run for 21 d. The chemicals used were NaCl, KNO<sub>3</sub>, HCl, H<sub>2</sub>SO<sub>4</sub>. These were left in dark, light and control. The emergence of radicle was considered as germination criteria<sup>14,15</sup>. The seed germination speed was calculated. The method of Djavanshir<sup>19</sup> were used for the determination of germination speed<sup>14,15,20,21</sup>. Anova Duncan test and Univariate variance analysis were used in the evaluation of germination percentage and rate.

## RESULTS AND DISCUSSION

*C. hausknetchii* is a perennial, 8-25 cm long, profusely branched with a woody rootstock, numerous sterile and fertile shoots forming a loose cushion, stems erect, simple, densely foliate. Leaves adpressed grey-tomentose, basal and lower entire or mostly pinnatifid with 2-3 pairs of short segments, median and upper similar or pinnatilobate to dentate, sessile and shortly decurrent, segments (lobes) ending in a yellow 2-10 mm spinule. Involucre 20-22 × c.13 mm, nearly cylindrical, arachnoid-floccose. Appendages a 10-20 mm spine, with (1-) 2-4 pairs of lateral 3-5 mm spinules near base. Flowers pink, marginal not radiant. Pappus 5-6 mm, inner row 1 mm.

*C. hausknetchii* populations are distributed between 1530 m and 1700 m altitudes. Population I grows at 1583 m (37°45' N, 38°39' E) on the north slopes of 10 degree inclination with 15 % coverage in a 870 m<sup>2</sup> area. Population II is found in 1650 m (37°40' N, 38°39' E) on the southwest slopes of 20 degree inclination with 25 % coverage in a 1400 m<sup>2</sup> area. Population III is found at 1716 m, (37°42' N, 38°39' E) with a 5 degree inclination and 10 % coverage in a 450 m<sup>2</sup> area. These populations in general grow on stony heaps comprised of crumbling dolomite limestone rocks. The soil samples taken from this site are rich in organic matter content and alkaline pH (Table-1). It grows on moderately basic calcareous habitats, rich in CaCO<sub>3</sub> and organic matter contents, but poor in nitrogen (Table-1). The soils show normal levels of Mg<sup>2+</sup> and K<sup>+</sup>, but Na<sup>+</sup> is below limits. On the dolomitic rocks its associates were *Hypocoum procumbens* L., *Thlaspi perfoliatum* L., *Cardaria draba* (L.) Desv., *Helianthemum salicifolium* (L.) Mill., *Ononis spinosa* L. subsp. *leiosperma* (Boiss.) Sirj., *Scabiosa argentea* L., *Senecio vernalis* Waldst. & Kit.

TABLE-1  
SOIL ANALYSIS OF *C. hausknetchii* POPULATIONS (0-30 cm)

Soils characters	Population I	Population II	Population III
pH	7.60	7.80	7.90
CaCO <sub>3</sub> (%) total	5.75	5.82	5.15
Organic Mat. (%)	4.90	4.20	3.80
P <sub>2</sub> O <sub>5</sub> (%)	75.00	45.00	29.00
N (%)	0.95	0.55	0.40
Ca <sup>2+</sup> (ppm)	9750	8500	7250
Mg <sup>2+</sup> (ppm)	1250	1110	950
K <sup>+</sup> (ppm)	7000	6000	4500
Na <sup>+</sup> (ppm)	35	40	20

It was observed that this area is under a serious pressure of grazing due to goats raised by the local population. The animals found near this area especially around Kahta are permanently grazing in the *C. hausknetchii* area. Stems of this species are without thorns and large leaves in vegetation period are deliciously consumed by the cattle heads. Since the capitulum are thorny they are not touched which is an advantage for the species. However, consumption of the plants in the vegetative period before capitulum mature is gradually reducing the population. The tourism too is effective in this connection and is threatening the populations of this species. The plants alongside the road to Nemrut peak are effected much by high traffic. The number of tourist is also increasing every year. Moreover, due to their attractiveness people always pick up the flowers thus adding to the present threatening situation.

Germination rate in different salt concentrations and 8 h light-16 h darkness and 16 h light- 8 h darkness were similar. In each NaCl treatment group, germination percentage decreased at higher concentrations in 8 h light-16 h darkness and 16 h light- 8 h darkness photoperiod. Germination percentage in 0.5 % H<sub>2</sub>SO<sub>4</sub> treatment was same as in 1 % HCl. Germination percentages in different KNO<sub>3</sub> treatments were different from each other. Higher concentrations lowered the gemination percentages. There was no difference in 0.5 % KNO<sub>3</sub> and the two control group. 0.5 % NaCl and 2 % NaCl; 1 % NaCl and 2 % KNO<sub>3</sub>; 2 % NaCl and 0.5 % H<sub>2</sub>SO<sub>4</sub>; 0.5% HCl and 1 % HCl treatments showed similar effects on germination percentages. Germination percentages in different KNO<sub>3</sub> treatments were different from each other. No difference was found between 1 % KNO<sub>3</sub> and 24 h dark photoperiod treatments. It was observed that light intensity has a stimulatory effect on germination percentage.

According to univariate variance analyse of germination test results; treatments (0.5 %, 1.0 %, 2.0 % NaCl; 0.5 %, 1.0 %, 2.0 %, 3.0 % KNO<sub>3</sub>; 0.5 % H<sub>2</sub>SO<sub>4</sub>; 0.5 %, 1.0 % HCl concentrations and control (8 h light-16 h darkness and 16 h light-8 h darkness and 24 h dark) were statistically significant, photoperiod and photoperiod × treatment interactions were insignificant ( $p < 0.05$ ), (Table-2).

The treatments 0.5 %, 1.0 %, 2.0 % NaCl; 0.5 %, 1.0 %, 2.0 %, 3.0 % KNO<sub>3</sub>; 0.5 % H<sub>2</sub>SO<sub>4</sub>; 0.5 %, 1.0 % HCl concentrations and control (8 h light-16 h darkness and 16 h light-8 h darkness and 24 h dark), photoperiod and photoperiod × treatment interactions had significant impact on germination speed ( $p < 0.05$ ) (Table-3).

Population size of a plant, variations in its morphology and number of seeds produced are mostly affected by genetic changes during time, existence of predators and ecological factors<sup>22-24</sup>. The first critical stage in this connection is germination. The germination behaviour of *C. hausknetchii* seeds revealed that lower salt concentrations have no effect on the germination percentage. Higher salt concentrations decreased germination percentages (Table-2) and there was no germination in 3 % NaCl. This is in agreement with the findings reported by several other workers<sup>25</sup>. In each photoperiod, there was germination in low H<sub>2</sub>SO<sub>4</sub> (0.5 %) concentration. High H<sub>2</sub>SO<sub>4</sub> concentration hinders germination under all conditions as reported by Awodola<sup>26</sup> and Muhammed and Manusa<sup>27</sup> to KNO<sub>3</sub> is regarded as a germination stimulator<sup>28,29</sup>. In present studies, it was noticed that germination percentage in 0.5 % KNO<sub>3</sub> under both photoperiods was higher than 24 h darkness control group. However, excessive KNO<sub>3</sub> concentration hinders germination and has a negative correlation with germination percentage. As a result of fast urbanization and industrialization many environmental problems are faced by the plants<sup>30</sup>. One of these is acid rains which result in a decrease in the seed germination rate. In low HCl concentrations (0.5 %, 1.0 %) germination is at lowest levels, but no germination occurs at higher concentrations. HCl hinders the seeds germination in *C. hausknetchii*. *C. hausknetchii* can be accepted as an indicator species for serpentine habitats.

Nemrut Mountain is an important floristic area. It is one of the areas of major importance for *in situ* conservation of plant genetic diversity in Turkey. In the present study, an attempt has thus been made to collect information about the habitat, demography and biological relationships of this species. It will help in building up of an effective conservation program. The species is known only from type locality. Nearly a hundred mature specimens of this species were observed in an area and its occupancy is less than 10 km<sup>2</sup>. *C. hausknetchii* has been recorded in the critical (CR) category in the red data book of Turkey<sup>8</sup>. The present observations revealed that due to its

TABLE-2  
GERMINATION PERCENTAGE AND RATE OF *Centaurea hausknetchii* SEEDS UNDER TWO DIFFERENT PHOTOPERIODS\*

Concentration	Photoperiod I (8 h light-16 h darkness)				Photoperiod II (16 h light-8 h darkness)			
	GR	GR ± SD	G (%)	GP ± SD	GR	GR ± SD	G (%)	GP ± SD
NaCl	0.5 %	10.8 abcd	39.7 f	39.7 ± 3.3	12.1 ef	12.1 ± 1.3	44.2 g	44.2 ± 2.9
	1.0 %	11.5 cde	23 d	23.0 ± 2.2	11 de	11.0 ± 0.5	29.5 e	29.5 ± 1.3
	2.0 %	10.9 bcde	10.9 ± 0.7	15.5 c	13 f	13.0 ± 1.3	16.7 cd	16.7 ± 1.0
KNO <sub>3</sub>	0.5 %	11.9 de	11.9 ± 0.7	45.7 g	10.3 bcd	10.3 ± 0.2	52.2 h	52.2 ± 1.0
	1.0 %	12.0 e	12.0 ± 0.3	48.7 h	10.5 cd	10.5 ± 0.3	59.0 i	59.0 ± 2.6
	2.0 %	10.5 abc	10.5 ± 0.3	31.2 e	8.7 a	8.7 ± 0.2	37.7 f	37.7 ± 1.5
3.0 %	10.3 ab	10.3 ± 1.1	13.7 bc	13.7 ± 1.3	9.1 ab	9.1 ± 0.3	19.0 d	19.0 ± 1.4
H <sub>2</sub> SO <sub>4</sub>	0.5 %	9.7 a	9.7 ± 0.9	5.5 a	5.5 ± 1.3	9.3 abc	9.3 ± 1.7	12.5 ± 2.1
HCl	0.5 %	9.6 a	9.6 ± 0.9	11 b	11.0 ± 0.8	9.6 abc	9.6 ± 0.8	14.7 ± 1.0
	1.0 %	10.9 bcde	10.9 ± 0.7	6 a	6.0 ± 0.8	9.6 abc	9.6 ± 0.4	6.7 ± 1.0
Control (8 h d-16 h l)	HW	14.9 f	14.9 ± 0.4	58.5 i	58.5 ± 3.5	12.1 ef	12.1 ± 0.3	69.2 j
Control (24 h Dark)	HW	12.0 e	12.1 ± 0.9	42 f	42.0 ± 1.8	11 de	11.0 ± 0.2	50.2 ± 2.6

\*Within each column, means with the same letter are not significant; 95 % significant; Anova Duncan test.

TABLE-3  
UNIVARIATE ANALYSIS OF VARIANCE FOR GERMINATION PERCENTAGE (GP) AND RATE (GR)\*

Source	Germination percent (%)				Germination rate			
	Sum of squares	df	F	Sign.	Sum of squares	df	F	Sign.
Treatments	33320.6	11	811.50	0.000	126.851	11	19.800	0.000
Photoperiod	846.094	1	226.67	0.000	12.400	1	23.350	0.000
Treatments × Photoperiod	210.780	11	5.13	0.000	41.780	11	6.529	0.000

\*Within each column, means with the same letter are not significant; 95 % significant; Anova Duncan test.

limited distribution area and small population size this species is at high risk and could get extinct in near future if preventive measures are not taken. The biggest danger for this species is heavy grazing and picking of its attractive flowers. The data gained from field studies was evaluated according to IUCN categories (Criteria B1 B2)<sup>31</sup> and the critically endangered status has been proposed for the species. There is thus an immediate need for educating of locals and animal grazing should be restricted to the east and south slopes where this and other rare species are not distributed.

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