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STUDIES ON THE GERMINATION OF THREE ENDANGERED *CENTUREA* SPECIES

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Abstract

Centurea spicata, *C. fenzlii* and *C. kurdica* are widely used as ornamental plants in the Mediterranean and East Anatolian regions of Turkey. *C. spicata* occupies serpentine habitats in the macchia formations whereas *C. fenzlii* and *C. kurdica* cover basaltic and dolomitic habitats among the tragacanthic steppe. However, these species are facing a threat of extinction due to overexploitation. As such, an attempt was made to investigate the possibilities for their propagation. For this purpose, the seeds were placed for germination at optimum temperature (25 ± 1 ^{0}C) in different concentrations of KNO_3, HCl and NaCl. Two different photoperiods (8 hours light-16 hours darkness-I and 16 hours light-8 hours darkness-II) were used to mimic the natural conditions. Studies on the germination of three species undertaken to evaluate the possibilities to cultivate the native populations at a large scale showed that *C. spicata* did not germinate at 3 % NaCl, 2-3 % HCl and 3 % KNO_3 under both photoperiods, but germination rate was highest in 1% KNO_3 whereas germination percentage was higher in 0.5% KNO_3 and control group under photoperiod I. Under photoperiod II germination rate was highest in 1% NaCl and control. In the case of *C. fenzlii* the germination failed in 3 % NaCl, 2-3% HCl and 3 %KNO_3 under both photoperiods but under photoperiod I the germination rate was highest in control and 0.5% KNO_3, whereas the highest percentage germination was examined in control and 1% KNO_3. Under photoperiod II germination rate was highest in 1-2 %NaCl and control, and germination percentage was highest in control and 0.5% KNO_3. In *C. kurdica* under photoperiod I germination rate was highest in control and 1% KNO_3 but percentage germination was highest in control and 0.5 % KNO_3, whereas under photoperiod II rate of germination was highest in control and 1% KNO_3 and germination percentage in control and 0.5% KNO_3.

Key words: *Centaurea*, NaCl, KNO_3, HCl, Photoperiod.

Introduction

Seed germination and early seedling growth are critical stages for the establishment of plant populations in their natural habitats (Perez *et al.*, 1998; Grappin *et al*., 2000; Aiazzi *et al.*, 2002; Khan & Gulzar, 2003; Navarro & Guitián, 2003; El-Keblawy & Al-Rwai, 2005; Al-Khateeb, 2006). Many times light and temperature interact during this phase and this interaction may make seeds sensitive to light only at certain temperatures but not at others. The importance of light depends upon the size of the seed, its life form (annual or perennial) and the habitat of the species. Application of KNO_3 to the germination medium too influences germination during this step (David *et al.*,1999). Recent physiological models have implicated an interaction of light quality, soil nitrate, in light-affected germination (Hilhorst *et al.*, 1990; Grubisic & Konjevic, 1990). Nitrate-stimulated germination has also been associated with gap detection in grassland species where nitrate levels are lower beneath growing plants than bare soil (Baskin & Baskin,

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1998). In the Mediterranean- climate the presence of slightly elevated levels of nitrate in the soil could be a germination cue related to the winter rainfall regime (Goudey *et al*., 1988). In these soils, seedling survival over summer is enhanced when the seeds germinate below the soil surface.

The effects of NaCl, HCl and KNO_3 on the germination behaviour of *C. spicata, C. fenzlii,* and *C. kurdica* under two photoperiods was investigated. These species are widely distributed under arid environmental conditions in the Mediterranean and East Anatolian regions of Turkey. However, the populations of these species are facing a threat of extinction due to overexploitation of their highly attractive flowers. As such, studies on the germination of this species were carried out in order to evaluate the possibilities to start in-situ and ex-situ conservation to sustain and cultivate these species at a large scale.

Material and Methods

The dark coloured fully matured seeds of *Centurea spicata* Boiss., *C. fenzlii* Reichardt and *C. kurdica* Reichardt were collected during July, 2002. *C. spicata* seeds were taken from the natural populations distributed in the Macchia formations of Hatay area, 5 km from Erzin to Dortyol at an altitude of 180 m. Seeds of *C. fenzlii* were collected from Mus - Elazigi, 7th km., at an altitude of 1270 m., lying in the East Anatolian region of Turkey and those of *C. kurdica* from Bingol-Elazigi, 60th km., from an altitude of 1300 m. These were brought to the laboratory and stored in paper bags under dark conditions. In each experiment petri dishes with 9 cm diameter were used with 100 seeds per dish and four replicates were set up. In the centre of each dish a glass plate was placed covered with two layers of filter paper to prevent rotting of seeds due to excess water. Germination experiments were conducted at the predetermined optimum temperature of 25 ^{o}C ± 1 in the plant growth chamber (MLR-350 Model Sony, Japan). A constant temperature (25 ± 1 $^{\circ}$C) and a white light source (Photoperiod I - 8 hours light- 16 hours darkness and photoperiod II - 16 hours light- 8 hours darkness) were used all through the experiments. For each species, four main experimental series were set up in replicates with 100 seeds per dish. Seeds were placed for germinated in 0.5 %, 1%, 2% and 3% concentrations of NaCl, KNO_3 and HCl, a control group was also maintained using distilled water. Experiments were terminated on the 31st day, owing to a complete cessation in germination. Seeds were considered germinated when the radicle was touching the seed bed (Yücel, 2000).

Germination speed was calculated as a coefficient which takes into account germination percentage as well as total germination. This coefficient is thus different from the coefficients used by Paul (1972), Seaward (1981) and Yücel (2000).

Statistical analyses: The statistical model 1 was applied for investigating the effects of photoperiod and treatment on the germination rate and statistical model 2 was applied to evaluate the effects of species, photoperiod and treatments on the germination percentage.

$$Y_{ijkl}: \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\gamma)_{ik} + \varepsilon_{ijkl} \qquad (1)$$

Y_{ijkl} : observed value
μ : general population mean
α_i : effect of i^{th} species (i=1,2,3)
β_j : effect of j^{th} block (dose)
γ_k : effect of k^{th} treatment (k=1,2,3,4)
$(\alpha\gamma)_{ik}$: interaction effect between species and treatment
ε_{ijkl} : random error term

$$Y_{ijklm}: \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijklm} \quad (2)$$

Y_{ijklm} : observed value
μ : general population mean
α_i : effect of i^{th} species (i=1,2,3)
β_j : effect of j^{th} photoperiod (j=1,2)
γ_k : effect of k^{th} treatment (k=1,2,3,4)
$(\alpha\beta)_{ij}$: interaction effect between species and photoperiod
$(\alpha\gamma)_{ik}$: interaction effect between species and treatment
$(\beta\gamma)_{jk}$: interaction effect between photoperiod and treatment
$(\alpha\beta\gamma)_{ijk}$: interaction effect between species, photoperiod and treatment
ε_{ijkl} : random error term

Minitab for windows (Version 13.0) statistical package program was used to analyse the data.

Results and Discussion

C. spicata, C. fenzlii and *C .kurdica* are important ornamental species of Turkey possessing very bright, yellow and pink flowers and attractive capitulums. The germination and seedling growth are critical stages for the establishment of the plant populations of these three species, as such germination studies were undertaken to evaluate the possibilities which will enable us to sustain and cultivate the native populations at a large scale. The present studies revealed that in *C. spicata* germination (Fig.1a,b,c) did not occur in 3 % NaCl, 2-3% HCl and 3 % KNO_3 under both photoperiods, but germination rate was highest in 1% KNO_3 (12.2), whereas germination percentage was higher in 0.5% KNO_3 (61.9%) and control group (56.4 %) under photoperiod I. Under photoperiod II rate was highest in control (12.4) and 1% NaCl (11.7) germination percentage in 0.5% KNO_3 (39.7%) and control groups (38.3%). In the case of *C. fenzlii* the germination failed in 3% NaCl, 2-3% HCl and 3% KNO_3 under both photoperiods but under photoperiod I the germination rate was highest in control and 0.5% KNO_3 (11.9) whereas highest percentage germination was recorded in control (14.9), under photoperiod II germination rate was highest in 2% NaCl (13.0), 1% NaCl and control (12.1), and percentage germination was highest in control (69.2%) and 0.5% KNO_3 (52.2%). In *C. kurdica* under photoperiod I rate was highest in control (13.3) and 1% KNO_3 (11.7) but percentage germination was highest in control (39.3%) and 0.5 % KNO_3 (36.3%), whereas under photoperiod II germination rate was highest in control and 1% KNO_3(11.4) and percentage germination in control (39.3%) and 0.5% KNO_3 (36.3%).

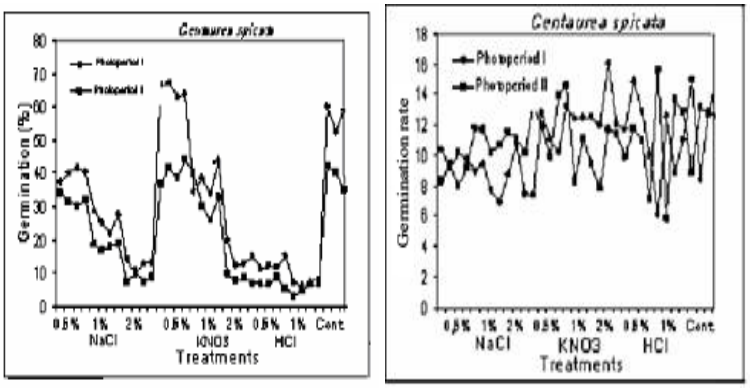

Fig. 1a. Germination percentage and germination rate in different treatments under two photoperiods ((8 h light-16 h darkness and 16 h light-8 h darkness) in *C. spicata*.

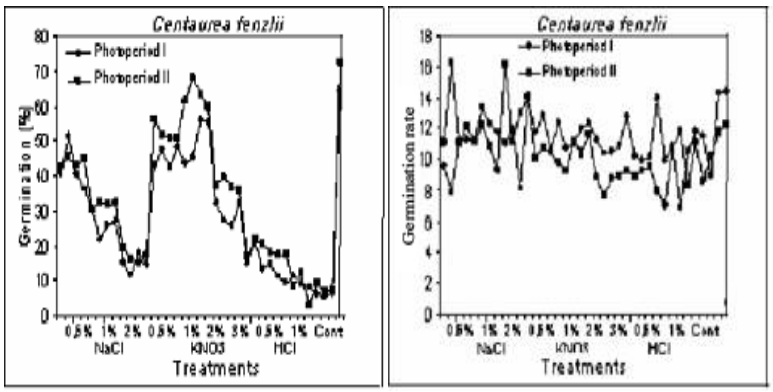

Fig. 1b. Germination percentage and germination rate in different treatments under two photoperiods ((8 h light-16 h darkness and 16 h light-8 h darkness) in *C. fenzlii*.

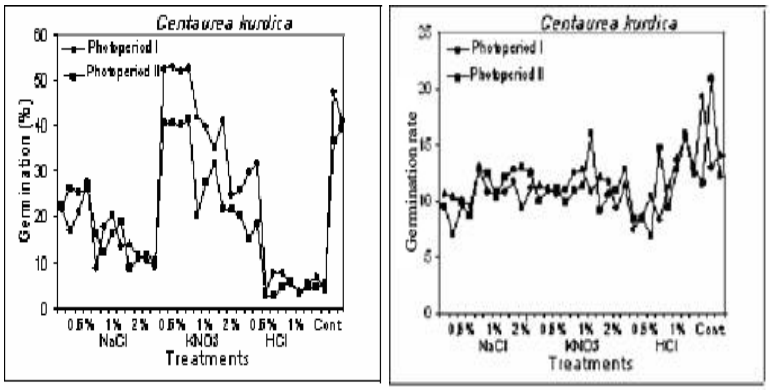

Fig. 1c. Germination percentage and germination rate in different treatments under two photoperiods ((8 h light-16 h darkness and 16 h light-8 h darkness) in *C. kurdica*.

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Table 1. Mean germination rate.

Species	Treatment			
	NaCl	KNO_3	HCl	Control
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
C.spicata	9.7±0.3 Bc	11.8±0.4 Aab	10.8±0.6 Bbc	10.0±0.2 Bc
C.fenzlii	11.8±0.4 Ab	10.5±0.3 Abc	10.0±0.4 Bc	13.3±0.3 Aa
C.kurdica	10.9±0.3 ABb	10.8±0.4 Ab	13.1±0.7 Aa	12.7±0.2 Aa

In the same species different small alphabets depict statistically significant differences among the tretments ($P<0.01$). In the same treatments different capital alphabets depict significant difference among the species ($P<0.01$).

Table 2. Interactions between the photoperiod (P) and different treatments (%).

Photoperiod	Treatment			
	NaCl	KNO_3	HCl	Control
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
P-I I	10.3±0.3Ac	11.5±0.2 Abc	11.4±0.6Abc	13.6±0.3Aa
P-II	11.3±0.3Aab	10.4±0.3Ab	11.2±0.7Aab	12.4±0.3Ba

In the same treatment different small alphabets depict statistically significant differences among photoperiods ($P<0.01$). In the same treatments different capital alphabets depict significant difference among the photoperiods ($P<0.01$).

Table 3. Interactions in relations to germination percentage between different treatments and photoperiod

Species	Photoperiod	Treatments			
		NaCl	KNO_3	HCl	Control
		$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
C.spicata	P- I	26±3.4 AcI	39.2±6.26AbI	9.8±0.9AdI	57.8±1.1AaI
	P-II	19.5±2.8BdII	26.8±4.2BcII	6.0±0.5AcII	39.30.3BaII
C.fenzlii	P- I	27.70.4AcI	35.2±4.2AbI	8.4±0.7AdI	58.8±2.4BaI
	P-II	30.8±3.4AdI	40.3±5.0AcI	10.1±1.5AeI	69.1±0.8AaI
C.kurdica	P- I	16.1±1.7AcII	39.9±3.1AaI	5.51±0.5AdI	45.10.9AaII
	P-II	17.1±1.9AcII	28.3±2.9BbII	4.1±0.4AdII	38.4±0.3BaII

In the same species and photoperiods different small alphabets depict statistically significant differences among treatment ($P<0.01$). In the same species and treatments different capital alphabets depict significant difference among the photoperiods ($P<0.01$). Same treatment and photoperiod different numbers (I,II) depict significant difference among the species($P<0.01$).

A perusal of the data on the effects of different treatments on the germination rate under photoperiod I and photoperiod II revealed that the germination rate follows the trend as control, KNO_3, HCl and NaCl under photoperiod I and control, NaCl, KNO_3 and HCl under photoperiod II. The data on the effects of different treatments on the germination percentage under photoperiod I and photoperiod II shows that in all three species not much affect is seen in this connection. The maximum germination percentage is seen in the control group and minimum in the HCl treatment group.

In conclusion, a comparison of NaCl and KNO_3 applications with control group reveals that statistically important differences exist in the mean germination rate of *C. spicata* with highest germination rate in the control. KNO_3 is used as growth-regulating

and germination-stimulating substance (Ozturk *et al*., 1993, Ozturk *et al*., 2006). It stimulates germination in many species (Ozturk *et al*., 1984), but can inhibit germination even at a low concentration in some species (Yucel, 2000). There are statistically important differences between NaCl, HCl and control groups as regards their effects on the germination rate of *C. fenzlii.* Similarly, differences between HCl and control are also important. In *C. kurdica* effects on the germination rate in the HCl and control groups are significant. A comparison of the germination rate under photoperiod I and photoperiod II shows that the highest germination rate occurs in the control group. As regards the percentage germination highest values are observed in control and the lowest in HCl.

Light and salinity interact during germination in a number of plants e.g., an increase in NaCl concentration may inhibit seed germination more in the dark than in light (Gul & Weber, 1999). A similar trend has been reported for some shrubs and forbs where inhibition is more substantial in dark than in light (Khan & Ungar, 1997). Also, both darkness and high salinity inhibit germination in *Limonium stocksii* (Zia & Khan, 2002). However, growth behaviour of majority of the species in particular crop plants is negatively affected by the saline environments (Ashraf *et al*., 2006; Ozturk *et al*., 2006). This might be the reason that *C. spicata, C. fenzlii* and *C. kurdica* completes its life cycle before the dry hot summer season starts in the steppes of Irano-Turanian plateau. We can conclude that species growing in the East Anatolia region of Turkey require a number of cues to stimulate germination. These cues are generally associated with winter when continuous moisture is available to ensure seedling establishment.

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