Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6[1] December 2016: 06-11

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Research of the Optimal Temperature Conditions for Germination of *Centaurea eriophora* achenes from Tessala Mount (western Algeria)

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ABSTRACT

Centaurea eriophora L. (Asteraceae) is an annual plant occurring in some Mediterranean regions. The present study was carried out to investigate germination behavior of achenes of Centaurea eriophora to constant temperature range. The Achenes which are collected from Tessala mount were exposed to constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 °C. temperatures below 10 and above 35 were unfavorable for germination. The highest FGP (85%) was obtained at 20 °C, which corresponds to the lowest IGD in the experiment (2 days). However, the FGP decreases above and below the 20 °C. The MTG and T_{50} are inversely proportional to FGP, they indicate how germination is rapid upon the time of the experiment.

Key words: *Asteraceae, Centaurea eriophora, germination, temperature, Tessala mount.*

Received 11.09.2016 Revised 02.10.2016 Accepted 09.10. 2016

INTRODUCTION

Centaurea eriophora L. (*Asteraceae*) is an annual plant, native to the western part from the Mediterranean region, in particular, south of the Iberian Peninsula, North West Africa and the Canary Islands (1). It grows on calcareous soils, on roadsides and in open arid spaces (2). In fact, *Centaurea eriophora* was considered as a quite rare plant in the north of Algeria (3), as well as the half of the Algerian flora is rare (4) (5). This native species grows also in Tessala Mount (western Algeria). And owing to the fact that this forest belt occupies of the remarkable ecological, geobotanic and socio-economic interests; it suffered with anthropic activities which remained the principal cause of the degradation of the flora.

The achenes of *Centaurea eriophora* L. are approximatively 4.0 – 4.5 mm long and 2.3 – 2.4 mm wide, brown and shiny color, with some hairs on their surface. An achene bears a lateral elaiosome at its base. The apex of the achene comprises toothed bristles of varying lengths, where the longest approach to 6-7 mm. The longer bristles of the pappus are flattened and curved where they emerge from the body of the achene (6). In fact, variation in seed mass can have a variety of implications for germination (7), but *C. eriophora* evinces no differences in germination between large and small achenes (2).

The germination is considered to be the most critical phase in the plant life cycle (8), and in the colonization process, which is basic to understand ecosystem dynamics (9). The rate of metabolic processes in all living organisms, and also the rate of development, is determined by temperature (10). In germination, the temperature range has been defined as having cardinal temperatures: minimum or base temperature (T_b), maximum temperature (T_{max}) below and above of which, the germination rate is zero and optimum temperature (T_{opt}) at which the germination rate is the highest (11). Temperature affects germination directly by fulfilling the specific germination requirements of different species, and it can also affect indirectly the rate of germination through its effects on changing the level of seed dormancy (12) (13) (14) (15). Changes occurring on temperature may affect a number of processes that control seed germinability, including membrane permeability and the activity of membrane-bound as well as the cytosolic enzymes (16).These characteristics interest not only the physiologists, but also ecologists, since it was possible to foretell the degree of success of the species, based on its capacity to produce achenes and distribute germination through time, allowing seedlings capture in the environment (17). Studies on the influence of temperature on the germination of achenes are essential to understand the ecological, physiological and biochemical aspects of the germination process (18) (19).

The purpose of this study was to clarify the germination characteristics of the freshly collected achenes of *C. eriophora* in a range of temperature, in order to determine the optimal temperature conditions to ensure a better germination for *C. eriophora* for the proper management in the restoration and conservation of existing populations in Tessala mount.

MATERIAL AND METHODS

Laboratory experiments were conducted to evaluate the effect of temperature on seed germination of *Centaurea eriophora* L. The achenes were collected in August 2015 from populations growing in Tessala mount (35°16'8.22"N, 0°46'46.26"O) at 719 m in north-western Algeria), which were stored for three months in a laboratory at an ambient temperature. The achenes kept in paper bags order to avoid contact with moisture until they ripen. Tessala mount, described by Dadach *and al.* (20), is a typical Mediterranean climate semi-arid region, characterized by irregular rainfall events and a harsh dry summer period. Annual precipitation is between 290 to 420 mm and Average monthly temperatures are between 9.4 °C and 26.6 °C.

In order to ensure the maturity of the achenes, the experiment was started after the collection in December 2015. However, by considering the fact that pathogen and non-pathogen organisms from the soil may affect the achenes, surface sterilization was made for 5 min in 10% sodium hypochlorite solution (NaOCl) then thoroughly rinsed two times by deionized water for 5 min to eliminate the chlorine effect. Germination tests were carried out in triplicate on 20 achenes placed inside closed plastic Petri dishes (90 mm of diameter) on an insured nontoxic commercial germination paper disk, and kept wet with tap water.

Germination experiments were performed in incubators (Memmert type) in darkness, with 8 different constant temperatures from very cold, cold fluctuating, fluctuating moderate and warmer, corresponding to 5, 10, 15, 20, 25, 30, 35 and 40° C. The achenes of *C. eriophora* were considered as germinated when a radicle appeared. Germination was scored every day for 30 days of experiments. Germinated seeds were removed from dishes and the number of new germinants was recorded

Seven parameters of germination were determined: cumulative germination, initial germination day (IGD), final germination day (FGD), initial germination percentage (IGP), final germination percentage (FGP), germination kinetics, mean time of germination (MTG) and the time to 50% germination (T_{50}).

MTG was calculated using the equation:

$$MGT = \sum \frac{(n_i \times d_i)}{N}$$

Where n_i is the number of achenes germinated until day i; d_i is the incubation period in days, and N is the total number of achenes germinated in the treatment (21). This means that MTG value is inversely proportional to the germination rate (22). T_{50} was calculated according to the following formula (23) (24):

$$T_{50} = \frac{[(\frac{N}{2} - n_i)(t_i - t_j)}{(n_i - n_j)} + t_j$$

Where N is the final number of germination and n_i, n_j cumulative number of achenes germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Data were subjected to One-way analysis of variance (ANOVA), to evaluate the effect of temperature on the individual parameters (IGD, FGD, IGP, FGP, MGT and T_{50}), and followed by the use of Duncan's multiple range test as *post hoc* to separate means. Data were analyzed using IBM statistics SPSS (23 version). The graphs have drawn with Excel Software.

RESULTS AND DISCUSSION

Our study was carried out to assess the effect of temperature on germination of species belonging to the *Asteraceae* family. Germination was significantly affected by temperature. The achenes of *C. eriophora* have been shown to be able to germinate at the temperature of 10 to 35 °C. These results are in agreement with those obtained by Zarghani *and al*. (25) who reported that the seeds of *Matricaria aurea, Cynara scolymus and Achillea millefolium* germinate in a wide range of temperature extending between 10 and 35 °C. The optimum constant temperature corresponding to the maximum germination was 20 °C with 85% (Figure 1); this value represents the highest FGP in the experiment (figure 4). Our data were supported by those obtained by Ruiz De Clavijo (2), as well as those including and describing the germination of the achenes of *Centaurea solstitialis* (26), *Centaurea virgata* (27) and *Centaurea calcitrapa*

(28). However, the germination was decreased above and below the optimum temperature. The *C. eriophora* achenes showed no germination neither at 5 and 40 °C. A such lack of germination that have been noted at low temperature (5 °C) was also reported in many species which can tolerate the same climatic conditions especially *Centaurea melitensis* (29) and *Centaurea diffusa* (30). Seed germination reduction at low temperatures is highly related to germination rate decrease, whereas at high temperatures is associated to high endogenous abscisic acid contents that inhibit the germination process (25) (31). The achenes of *C. eriophora* have presented a significant decline in germination at 15 °C (figure 4). Bain (29) reported that the peripheral achenes of *Centaurea melitensis* which have presented a lowering in germination at 15 °C may develop last in the season (May through June), and usually are the result of the plants last effort to produce achenes before full senescence. Moreover, these achenes may germinate even in stressful environmental conditions (limit rainfall and increase temperature).

The MTG also varied significantly in our study ($p < 0,05$) (Figure 3). The lowest MTG was noticed at 30 °C, whereas the highest MGT was obtained at 10 °C corresponding to a slower germination process. Bambo *and al.* (32) reported that low mean germination times indicating fast germination characterizes the species that can be established in the environment as quickly as possible. The temperature has a significant effect on IGD ($p < 0.05$) (table 1); however, the first time of germination was noted at 20 °C in less than 48 hours. That is expressed by the fast imbibition mechanism which happens to the achenes. In fact, this process corresponds to the succession of three phases. Firstly, a rapid water uptake, then, a little change in water uptake is representing a plateau phase, and finally a subsequent increase in water content coincident with radicle growth (33) (34). In opposition, the longest IGD was observed at both 10 and 15 °C with a delay of 4 days (Table 1). Which means that the germination of the achenes of *C. eriophora* start after a long period of latency at cold temperatures. Furthermore, the longest time to reach 50% of germination (T_{50}), which is significantly varied ($p < 0,05$), was noted at 10 °C with 5.26 days, when 2.15 days considered as the lowest T_{50} was noted at 30 °C (Table 1). Some Asteraceae species such as *Conyza canadensis* (L.) Cronquist and *Galinsoga parviflora* Cav. have been shown to reached 50% of germination in 5 days at cold temperatures (35). Otherwise, the IGP was not affected significantly by temperature ($p > 0.05$), where the highest value of IGP was found at 35 °C. It means that there is no relationship between the temperature and *C. eriophora* ability to germination in the initial days.

On the other hand, the interactions between these parameters were firstly analyzed in this study using the polynomial regression analysis. We found that there is a very low correlation between the FGP and the temperature ($R^2 = 0.0038$) (Figure 1) (Figure 2). A strong significant correlation was observed in the relationship between mean germination time (MGT) and temperature range, which the regression coefficient R^2 corresponding to 0.95 whereas $R^2 = 0.94$ represents the strength of the relationship T_{50} / temperature range (Figure 3). The MGT and the T_{50} calculated using formulas which are described in the data analysis showed that they decreased with an increase of IGP and both of them increase when FGP decrease. These results revealed that the MTG and T_{50} are inversely proportional to FGP (Figure 4).

Table 1 : germination characteristic variables of *Centaurea eriophora* achenes in response to different temperatures (means ± SE, N=3)

Temperatures (°C)	IGD (days)	FGD (days)	IGP (%)	FGP (%)	TMG (days)	T_{50} (days)
5	0^{a}	0^{a}	0^{a}	0^{a}	-	-
10	4 ± 1^{d}	14.33 ± 2.51^{b}	10 ± 5^{ab}	31.66 ± 2.88^{b}	6.64 ± 1.63^{b}	5.26 ± 0.64^{c}
15	4 ± 0^{d}	24.33 ± 2.08^{c}	26.66 ± 5.77^{bc}	30 ± 5^{b}	4.25 ± 0.30^{a}	3.56 ± 0.06^{b}
20	2 ± 0^{b}	25.66 ± 0.57^{c}	18.33 ± 7.63^{abc}	85 ± 0^{e}	3.30 ± 0.47^{a}	2.75 ± 0.37^{ab}
25	2 ± 0^{b}	25.66 ± 0.57^{c}	20 ± 11.54^{bc}	68.33 ± 2.88^{d}	3.34 ± 0.79^{a}	2.86 ± 1.01^{ab}
30	2 ± 0^{b}	25.66 ± 0.57^{c}	26 ± 7.63^{bc}	40.33 ± 10.40^{c}	2.94 ± 0.15^{a}	2.15 ± 0.48^{a}
35	3 ± 0^{c}	26.33 ± 1.15^{c}	31.66 ± 6.07^{c}	36.66 ± 12.58^{b}	3.95 ± 1^{a}	2.62 ± 0.21^{ab}
40	0^{a}	0	0^{a}	0^{a}	-	-
F value	57.85**	249.71**	4.601	69.25**	7.06*	11.78**

Different lowercase letters (column) show significant differences between the averages, F-probabilities are indicated by symbols: * significant differences at $p < 0.05$, **significant differences at $p < 0.01$, according to Duncan multiple comparisons test

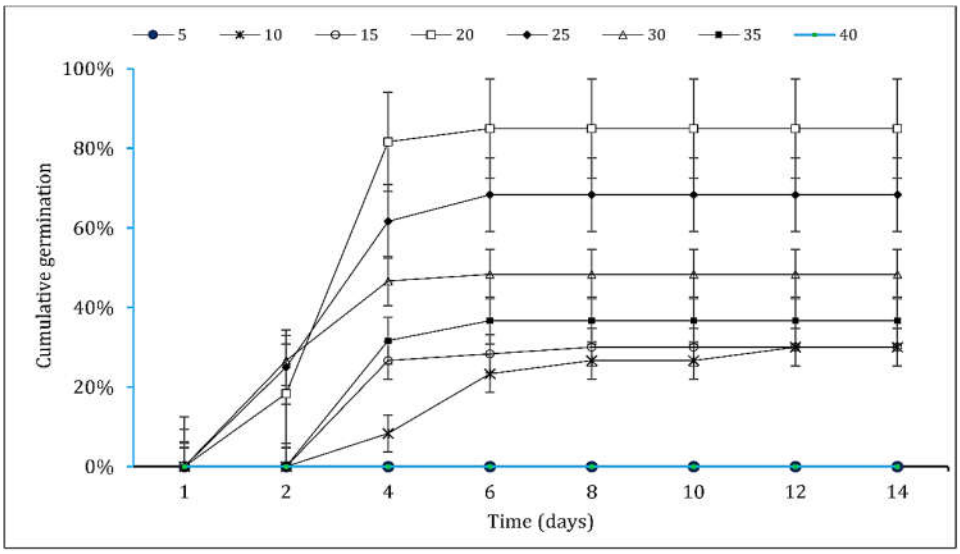

Figure 1: Cumulative germination percentage in response to different temperatures
Bars represent ± Standard Error, (n = 3). Confidence interval were calculated at the threshold of 5%

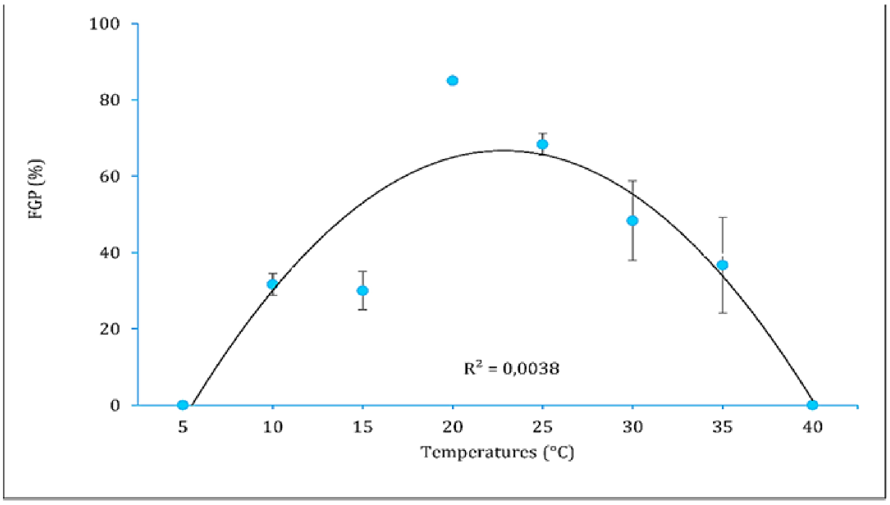

Figure 2 : Polynomial plot for final germination percentage (FGP) at various temperatures regimes
Bars represent ± S.E, (n = 3). The confidence intervals were calculated at the threshold of 5%

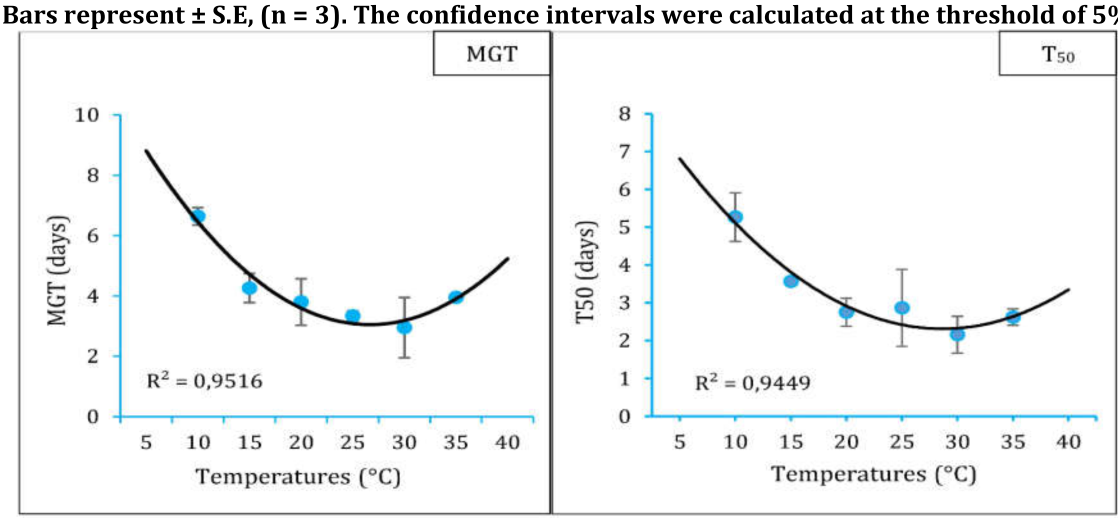

Figure 3: Polynomial plots for mean germination time (MGT) and T_{50} at various temperatures regimes
Bars represent ± S.E, (n = 3). The confidence intervals were calculated at the threshold of 5%.

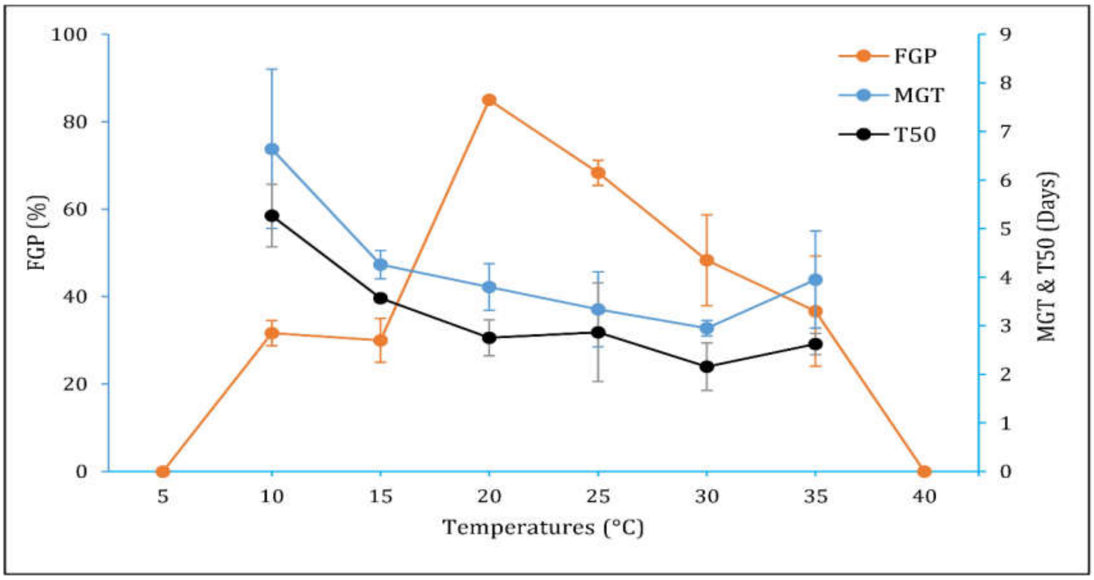

Figure 4 : Effect of different temperatures on final germination percentage (FGP), mean germination time (MGT) and T_{50}.
Bars represent ± S.E, (n = 3). The confidence intervals were calculated at the threshold of 5%.

CONCLUSION

The study here in lead us to suggest that *Centaurea eriophora* does not have problems of reproductivity related with germination under the temperature factor. The ability of germination of *Centaurea eriophora* achenes in different studied temperatures may reflect a possibility of germination whatever the environmental conditions to which they are submitted. Whereas, the main threats for this species are likely to be anthropogenic activities like excessive pasture and overgrazing that reduce the forest remnants; where seedlings can establish and grow under the stressful Mediterranean climate. *Ex situ* conservation activities like germplasm storage in seedbanks provide a basis for future habitat restoration efforts and conservation of this rare species.

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CITATION OF THIS ARTICLE

Amar Bouker, Zoheir Mehdadi, Ali Latreche, Boubaker Saidi, Abd Latif Adjouj, Mohammed Dadach. Research of the Optimal Temperature Conditions for Germination of *Centaurea eriophora* achenes from Tessala Mount (western Algeria).Bull. Env. Pharmacol. Life Sci., Vol 6[1] December 2016. 06-11