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CARDINAL TEMPERATURES FOR GERMINATION OF THE MEDICINAL AND DESERT PLANT, *Calotropis procera*[1]

Temperaturas Cardeais para a Germinação da Planta Medicinal e do Deserto ***Calotropis procera***

TAGHVAEI, M.[2], SADEGHI, H.[2], and KHAEF, N.[2]

ABSTRACT - *Calotropis procera*, Apocynaceae, is a wild perennial shrub that originated in the Persian deserts. It is known to provide key resources in degraded ecosystems to about 80 animal species. *C. procera* is regenerated by seed and produces lots of small seeds that are dispersed by wind; nonetheless, its density is very low. The purpose of this study is to estimate the cardinal temperatures including the base, optimum, and maximum temperatures of *Calotropis procera* looking at two different ecotypes in the Iranian desert. The germination behavior of *C. procera* seeds was tested at temperature regimens of 0, 5, 10, 15, 20, 25, 30, 35 and 40 ^{o}C and was analyzed using linear regression models. The rate of germination increased between base and optimum thermal conditions, and decreased between optimum and maximum thermal conditions. The base, optimum and maximum temperatures for germination of *C. procera* seeds were estimated at 19.10, 30.75 and 47.80 ^{o}C for the Fars and 20.00, 31.82 and 49.69 ^{o}C for the Zahedan desert, respectively. Temperature and germination were rated to determine the seeding dates of the *C. procera.* Overall, cardinal temperatures for germination were dependent on local climate characteristics for the range of adaptations in plant growth of the given species**.**

Keywords: base temperature, optimum temperature, linear regressions.

RESUMO - Calotropis procera (Aiton), Apocynaceae, é um arbusto perene silvestre originado dos desertos persas. É conhecido por atuar como provedor de recursos-chave em ecossistemas degradados para cerca de 80 espécies animais. É regenerado por sementes e produz grande quantidade de pequenas sementes, que são dispersas pelo vento; no entanto, a sua densidade é muito baixa. O objetivo deste estudo é estimar as temperaturas cardeais, incluindo as temperaturas base, ótima e máxima, de ***Calotropis procera****, olhando para dois ecótipos diferentes no deserto iraniano. O comportamento de germinação de sementes de C. procera foi testado em regimes de temperaturas de 0, 5, 10, 15, 20, 25, 30, 35 e 40 °C e analisado utilizando modelos de regressão linear. A taxa de germinação aumentou entre as condições térmicas base e ótima e diminuiu entre as condições térmicas ótima e máxima. As temperaturas base, ótima e máxima para a germinação de sementes de* ***C. procera*** *foram estimadas em 19,10, 30,75 e 47,80 ºC para o deserto Fars e em 20,00, 31,82 e 49,69 ºC para o deserto Zahedan, respectivamente. Temperatura e germinação foram avaliadas para determinar as datas de semeadura de* ***C. procera****. De modo geral, as temperaturas cardeais para a germinação são dependentes de características climáticas locais para a gama de adaptações no crescimento das plantas das espécies dadas.*

Palavras-chave: temperatura base, temperatura ótima, regressões lineares.

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[2] Department of Natural Resources and Environmental Engineering, College of Agriculture, Shiraz University, Shiraz, Iran, <sadeghih@shirazu.ac.ir>.

INTRODUCTION

Calotropis procera, Apocynaceae, is a wild perennial shrub that originated in African, Indian and Persian deserts (Neto et al., 2013). In these areas, the average annual rainfall is between 30 and 200 mm, or even less. In Asia, *Calotropis* spp. is distributed from the Mediterranean to the Africa coast. In Iran, it is mainly distributed in the Fars province (Lamerd) and can also be found in the Sistan–Balochestan province (Zahedan). Iran is a country located in the belt of the arid and semi-arid regions of the Earth. Approximately 60% of Iran is classified as arid and semi-arid. In arid and semi arid lands, evaporation rises and rainfall is almost nonexistent during summer. (Lin & Chen, 1978). The *Calotropis* spp. plays a key role not only in restoring the degraded land but also in sustaining these areas. It is known to provide key resources in degraded ecosystems to about 80 animal species including two predispersal insect seed predators, which solely depend on the plant for completion of their life cycle. The plant is well known for its abundant and continuous latex production that is mainly recovered in the green parts of the plant. The latex has been reported to comprise chitinases, proteinases and anti-oxidative enzymes (Freitas et al., 2007). Further studies have shown that laticifer proteins are involved in the plant's defense against crop pests (Ramos et al., 2007; Ramos et al., 2010). Several reports in literature indicate that the plant contains various classes of secondary metabolites, including flavonoids, cardioactive glycosides, triterpenoids, alkaloids, resins, anthocyanins, tannins, saponins and proteolytic enzymes (Neto et al., 2013). *C. procera* is regenerated by seed and produces lots of small seeds that are dispersed by wind; nonetheless, its density is very low. Establishment of the species in new sites depends on seed dispersal, germination and the establishment of seedlings. Seed germination is affected by light, temperature, and a host of other environmental factors (Bewley & Black, 1994). Temperature during seed imbibitions has been found to influence the germination rate and the ultimate percentage of germination (Flores & Briones, 2001). Since as early as 1860, it has been recognized that three cardinal temperatures (minimum or base temperature T_b, optimum temperature, T_{opt} and maximum temperature, T_{Max}) describe the temperature range over which the seeds of a particular species can germinate (Bewley & Black, 1994). Seeds of each species possess the capacity to germinate within a defined temperature range, which is referred to as the cardinal temperature (Alvarado & Bradford, 2002). Cardinal temperatures for germination of most crop plants tend to be similar to those of normal vegetative growth (Gardner et al., 1985). However, for some species, cardinal temperatures for germination may differ from those of root or shoot elongation (Lawlor, 1987). Finally, the cardinal temperature (minimum or base, optimum and maximum temperature) is the temperature range within which the seeds of a particular species can germinate. The minimum cardinal temperature is the lowest temperature at which crop growth can occur; this temperature is referred to as the base temperature, and no growth occurs below this temperature. The optimum cardinal temperature is the temperature at which crop growth and performance are at their maximum. The maximum cardinal temperature is the highest temperature at which plant growth can occur (Alvarado & Bradford, 2002). Some methods have previously been developed to describe species growth data (e.g. germination percentage, germination rate) in response to temperature, but amongst those methods, the germination rate method is the most specifically salient to temperature (Covell et al., 1986). A great deal of research has been done in attempt to understand these relations. Regression analysis is generally recognized as the best statistical tool for the investigation of relationship among these variables. The data collected at sub-optimal and supra-optimal temperatures was used to construct two linear regressions to describe the increases and decreases in the germination rate at sub-optimal and supra-optimal temperatures, respectively by Taghvaei et al. (2010). The optimum temperature is the temperature at which these two lines intersect (Covell et al., 1986). Roberts (1988) previously described a model elucidating the relationship between germination rate and temperature. The influence of temperature on germination rate and thermal time in plant species, such as the common crupina (*Crupina vulgaris*) (Shafii & Price, 2001), Himalayan elm (*Ulmus wallichiana*) (Phartyal et al., 2002) have been previously evaluated by many different researchers,

Khaef et al. (2010) reported that no significant differences were observed between light and darkness in terms of the seed germination rates of *C. procera*, and the highest percentage of germination was observed at 30 °C under both light and dark condition. However, no research has yet been conducted regarding the germination responses of *C. procera* at cardinal temperature. Thus this research was conducted to evaluate the relationship between the cardinal temperatures including base, optimum, and maximum temperatures of *Calotropis procera* from two different ecotypes in the Iranian desert.

MATERIAL AND METHODS

Plant material

Mature seed lots of *C. procera* were collected from two arid areas: the Fars Province (Lamerd) dune desert (28°43'26" N, 53°23'19" E) and the Sistan-Balochestan Province (Zahedan) dune desert (29°29'47" N 60°51'46" E). Both have different climates (Tables 1 and 2) and are located in the south and southeast of Iran, respectively (Figure 1). After eliminating humidity, the seeds were stored in bags in a refrigerator (at 5 °C) until the beginning of the experiment. This study was conducted at the Department of Desert Region Management at the College of Agriculture of the Shiraz University in Iran.

Four replications of 50 seeds of two provenances were sown at 0, 5, 10, 15, 20, 25, 30, 35 and 40 °C on Whatman No.1 filter paper, in Petri dishes (90 mm diameter) under light conditions for germination test (khaef et al., 2010). The filter paper was moistened with 5 mL of distilled water, allowing about half of the seeds to be immersed in the solution. During this experiment, lost water was replaced when necessary (Sadeghi et al., 2012).

The seeds were considered to have germinated when the emerging radical was over 2 mm long (ISTA, 1999). The number of germinated seeds was recorded on a daily basis. After seven days, the final number of

Table 1 - Average maximum and minimum temperature and monthly average of total precipitation of the desert area in the Fars province

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Maximum temperature (°C)	21.5	28.5	28.5	31.4	39.0	44.3	45.3	44.1	42.2	38.5	31.5	24.5	34.9
Minimum temperature (°C)	6.5	8.5	15.5	15.7	20.5	24.2	25.5	26.0	23.5	18.2	13.3	7.5	17.1
Mean temperature (°C)	14.0	17.5	17.0	23.5	34.2	34.2	35.5	35.0	33.2	24.4	22.4	16.0	25.6
Precipitation (mm)	63.6	43.5	10.8	24.4	0.5	0.15	0	0.25	0	0.6	11.5	99.2	254.5

Table 2 - Average maximum and minimum temperature and monthly average of total precipitation of the desert area in the Zahedan province

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Maximum temperature (°C)	20.1	26.0	27.3	33.4	41.0	45.0	47.0	43.5	41.1	37.6	33.6	26.3	35.1
Minimum temperature (°C)	5.8	7.3	13.5	17.8	22.3	25.8	27.9	29.4	21.7	16.4	14.9	9.7	17.7
Mean temperature (°C)	12.0	16.0	20.4	25.6	31.6	35.4	37.4	36.4	31.4	27.0	24.2	18.0	26.4
Precipitation (mm)	55.6	45.5	14.8	21.4	1.5	0.85	0.1	0.95	0	0.5	9.8	77.1	228.1

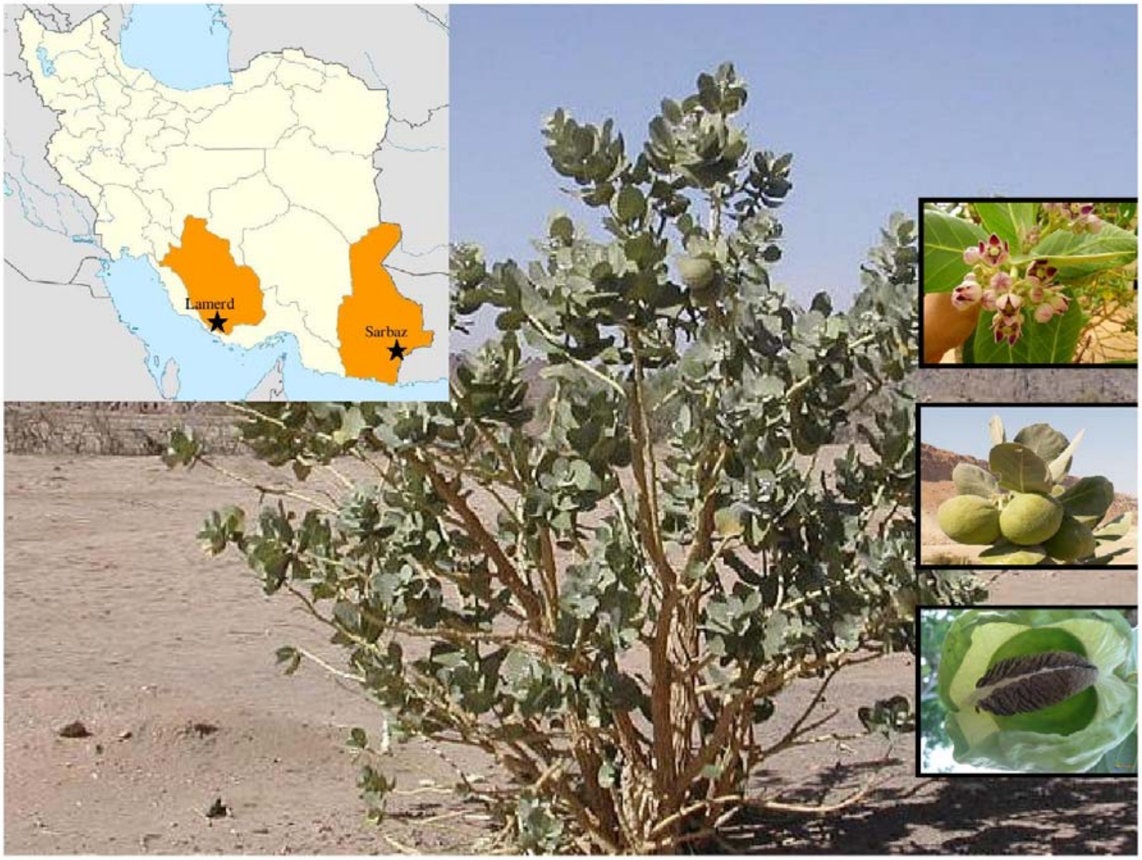

Figure 1 - Map of the study area and location of experiment.

germinated seeds was calculated, as well as the percentage of germination. The mean time to full germination was calculated in accordance with the equation developed by Ellis & Roberts (1981). The germination rate was calculated by the inverse of mean time to full germination (Tobe et al., 2000).

$$MTG = \Sigma (ni.ti)/\Sigma n$$

MTG: Mean time to full germination; n: number of seeds newly germinating at time 't'; GR= 1/MTG; GR: germination rate; t: number of days from sowing.

Cardinal temperature was calculated on the basis of the responses of the germination rate to temperature (Taghvaei et al., 2010). An intersecting–line model (Al- Sobhi et al., 2006) was employed. The T base and T maximum were derived from the intersection of each regression line with the abscissa, and the T optimum was calculated from the intersection of the two linear regression lines of the germination rate at sub-optimal and supra-optimal temperatures (Covell et al., 1986).

Data analyses

Data was checked for normality and then analyzed using MSTATC statistical software (MSTATC Inc., East Lansing MI, USA). Treatment means were separated by Duncan's test in cases where the F value of the treatments was significant at a probability level of 0.05 and 0.01.

RESULTS AND DISCUSSION

Fars dune desert seed source

The seeds germinated more rapidly at 30 ^{o}C than at other temperature treatments, during the first 120 hours and reached a germination peak (Figure 2), and seed

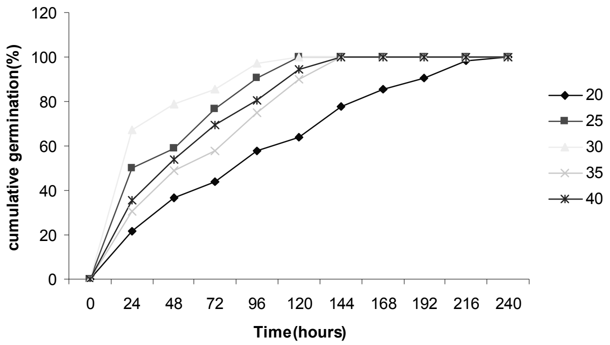

Figure 2 - The trend of cumulative germination response to temperature at 24 hours intervals at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C. Each point represents the mean of 4 replications (50 seeds).

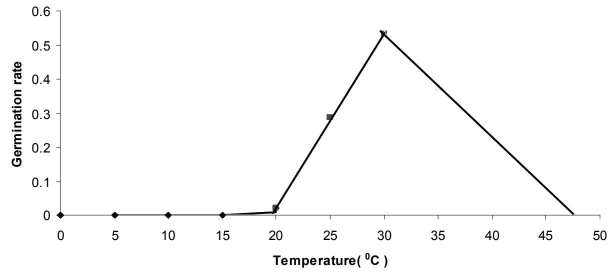

Figure 3 - *Calotropis procera* germination rate response to temperature in the Fars dune desert seed source.

germination was slower at 20 °C than at other temperatures. The germination percentage of the seeds at 30 °C reached 100% after 120 hours, but at 20 °C the germination percentage of the seeds reached only 60% after 120 hours. Despite this, the germination percentage reached a level of 100% after 240 hours at 20 °C. Seeds germination was slower at 25 °C, 35 °C and 40 °C than at 30 °C, but the germination percentage of seeds reached 100% after 240 hours for all three temperatures. The germination rate increased along with increasing temperature.

The highest seed germination rate (0.5) was obtained at 30 °C. The germination rates were 0.1 and 0.5 at 22 °C and 26 °C, respectively, and decreased to 0.3 at 35 °C (Figure 3). The highest positive slope of germination was noted at 30 °C; however, it was reduced at 35 °C (Table 3). The fitted regression line for the germination rate evidenced a positive slope between 20 °C and 30 °C and a negative slope between 30 °C and 40 °C.The intersection of fit linear regression between 20 °C and 30 °C and between 30 °C and 40 °C with the abscissa showed a T base of 19.1 and a *T. maximum* of 47.8, respectively (Figure 3 and Table 3). The intersection of two linear regression lines of the germination rate at sub-optimal and supra-optimal revealed the optimal temperature of 30.75 °C. (Figure 3 and Table 3).

Zahedan dune desert seed source

The germination rate differed significantly between temperature treatments (p<0.01). In the first 120 hours, the response of the germination to temperature differed completely. The seeds germinated rapidly at 30 °C and then reached their peak of germination (Figure 4), and the percentage of germinated seeds was lower at 20 °C than at 25 °C and 30 °C. The germination percentage of seeds reached 100% at 30 °C after 96 hours, but at 20 °C the germination percentage of seeds was 60% after 120 hours, and the germination percentage of seeds reached 82% at 25 °C after 96 hours.

The germination rate indicated an increasing trend from 20 °C to 30 °C and then the germination rate decreased abruptly with increases in temperature. The highest and lowest germination rates were noted at

Table 3 - Equation calculated cardinal temperature for *Calotropis procera* seeds

Seed source	Equations <op	Equations >op	T_b (°C)	T_{opt} (°C)	T_{Max} (°C)
Fars	GR = 0.051T- 0.998	GR = -0.0302T+ 1.436	19.10	30.75	47.80
Zahedan	GR = 0.039T- 0.759	GR = -0.0199T+ 0.985	20.00	31.82	49.69

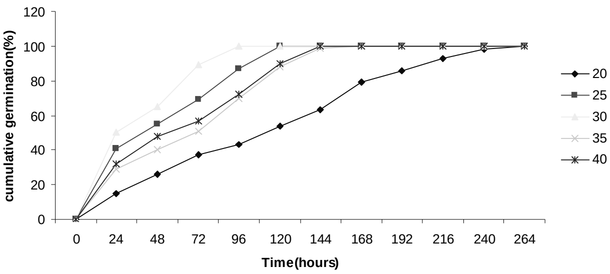

Figure 4 **-** The trend of cumulative germination response to temperature at 24 hours intervals.

30 °C and 35 °C, respectively (Figure 5). The germination rate was 0.21 at 25 °C and reached 0.40 at 30 °C, and then decreased to 0.30 at 35 °C (Figure 5). The drawn-to-fit linear scale to cumulative normal distribution evidenced a positive slope from 20 °C to 30 °C and a negative slope from 30 °C to 35 °C. The intersection of fit linear regressions between 20 °C to 30 °C and between 30 °C to 35 °C with the abscissa showed a T base of 20 °C and a *T. maximum* of 49.69 °C respectively (Table 3). The intersection of the two linear regression lines of the germination rate at sub-optimal and supra-optimal temperatures revealed the optimal temperature at 31.82 °C (Table 3).

Seed germination is a critical stage that is profoundly affected by environmental factors, particularly by cardinal temperature (minimum, optimum, and maximum temperatures). Our results showed that *Calotropis procera* seed germination is affected by temperature. Many authors have previously reported this in their studies (Phartyal et al., 2002).

Seed germination started at 22 °C and the germination rate increased with increasing temperatures, showing a positive linear relationship between tempera-ture and germination rate from 20 °C to 30 °C. Then, germination rate decreased with increasing temperatures so a negative linear relationship was detected from 30°C to 45°C (Figure 2 and 3). Taghvaei et al. (2010) reported similar results about the germination rate responses of *Haloxylon aphyllum* Khaef et al. (2010) reported that the optimal temperature for the germination of *Calotropis procera* was 25 °C, and pointed out that germination increased until 35 °C and then decreased. When fitting the linear regression of the point germination rate at sub-optimal and supra-optimal temperatures, the base temperature and maximum temperature for *C. procera* were ultimately revealed. Shafii & Price (2001) reported using the cardinal temperature of common crupina (*Crupina vulgaris*) with a linear regression of the germination rate and the sub-optimal and supra-optimal temperatures.

Hardegree (2006) predicted the cardinal temperatures of germination for four rangeland grass species using a regression method. Taghvaei et al. (2010) generated a model for predicting the cardinal temperature of milkweed (*Calotropis procera).* The cardinal temperatures for germination have been determined via the use of germination rate for different plants (Rizzardi et al., 2009).

Cardinal temperature was affected by seed source. Lawrence (2001) reported that response to temperature in germination stage depends on the species, variety, growing region, seed quality, and the length of time to harvesting. As a general rule, temperate-region seeds require lower temperatures than tropical region seeds, and wild species have lower temperature requirements than domesticated plants (Ali et al., 2008). The base temperature differed between the two seed provenances. The base temperatures were 20 °C and 19 °C in the Zahedan and Fars desert regions, respectively (Table 3). The range in base temperatures between two seed lots of milkweed (*Calotropis procera)* was 0.62 °C and 1.76 °C, but was not statistically significant, although it may be biologically significant (Taghvaei et al., 2010).

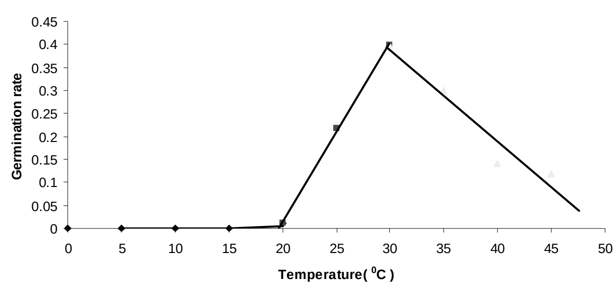

Figure 5 **-** *Calotropis procera* germination rate response to temperature in the Zahedan dune desert seed source.

The maximum temperature differed between the two seed provenances. The maximum temperatures were 49.69 °C and 47.8 °C in the Zahedan and Fars desert regions, respectively (Table 3). The optimal temperatures were 31.82 °C and 30.75 °C in the Zahedan and Fars desert regions, respectively (Table 3). Ali et al. (2008) reported the variation in base and maximum temperature requirements for germination among different wheat (*Triticum aestivum*) genotypes.

In conclusion, cardinal temperatures for germination are dependent on local climate characteristics for the range of adaptations of species. Cardinal temperatures help us determine germination timing and favorable conditions for the early growth and development of seedlings. Although rainfall and moisture form one of the inhibiting environmental factors in the desert, the temperature is the most important factor for germination and planting dates. Temperature had a significant effect on the germination of seeds taken from both provenances.

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