



## EXPERIMENTAL PAPER

### Cardinal temperatures for germination of *Salvia leriifolia* Benth.

MAJID DASHTI<sup>1,2\*</sup>, MOHAMMAD KAFI<sup>1</sup>, HOSSEIN TAVAKKOLI<sup>2</sup>, MAHDI MIRZA<sup>3</sup>

<sup>1</sup>Ferdowsi University of Mashhad  
Department of Agronomy  
Mashhad, Iran

<sup>2</sup>Khorasan-e-Razavi  
Agricultural and Natural Resources Research Center  
Mashhad, Iran

<sup>3</sup>Research Institute of Forest and Rangeland  
Medicinal Plant Research Branch  
Tehran, Iran

\*corresponding author: e-mail: Majiddashti46@gmail.com

#### Summary

The focus of this study is based on the examination of the germination traits and the development of thermal models of the medicinal plant *Salvia leriifolia* Benth. A laboratory experiment was carried out at constant temperatures ranging from 0 to 35°C, at 5°C intervals in a completely randomized design with eight replications. To describe the germination rate response to temperature, three regression models, namely Intersected-Lines (ISL), Quadratic Polynomial (QPN) and Five-Parameters Beta (FPB) were used. The highest Germination Percentage (GP) (92.8%) occurred in 15°C, but GP in the range of 10–25°C was not significant ( $p \leq 0.05$ ). The germination process stopped at 0°C and at above 30°C. The results indicated that the highest Germination Rate (GR), the lowest Mean Germination Time (MGT) and also times to 50% germination ( $D_{50}$ ) were obtained at 20°C. Seeds did not reach to their 50% germination level in temperatures higher than 25°C. The FPB model had the best realistic estimation for cardinal temperatures. Based on models estimation, Base ( $T_b$ ), Optimum ( $T_o$ ) and Ceiling ( $T_c$ ) temperatures were in the ranges of (1–1.9°C), (18.1–20.8°C) and (34.5–38.7°C), respectively.

**Key words:** cardinal temperatures, medicinal plants, *Salvia leriifolia* Benth., regression models

## INTRODUCTION

*Salvia* is the largest genus of plants in the mint family, *Lamiaceae*, with approximately 700–900 different species in the world [1, 2]. Fifty-eight species of the genus *Salvia* are found in Iran, of which 17 are endemic including *Salvia leriifolia* Benth. (with vernacular names such as Noroozak, Jobleh, Cheble). It was introduced in Flora Iranica in 1982 as a perennial herbaceous plant, growing in Rocky Mountains and desert rangelands in cold-semi arid to cold-arid climate, exclusively in south regions of Khorasan and Semnan provinces in Iran, and small parts of Afghanistan [3].

In recent years, various medicinal properties of this plant, such as the attenuation of morphine dependence, hypoglycemic, anti-nociceptive, anti-inflammatory, antioxidant, anti-ischemia, anti-convulsion, anti-ulcer, antibacterial and anti-mutagenic activities were evaluated [4]. It was shown for the first time that leaf and seed extracts of *S. leriifolia* significantly prolonged the survival time following hypoxic stress in mice [5]. Also the anti-ischemic effect of aqueous extracts of *S. leriifolia* leaf and seed in rat hippocampus was reported [6]. The results of another investigation indicated that pretreatment with aqueous and alcoholic extracts of *S. leriifolia* root, significantly reduced the elevated concentration of lipid peroxides in rat hippocampus following ischemic-reperfusion injury [7].

It has also been shown that the alcoholic extract of *S. leriifolia* leaf attenuates the withdrawal syndrome (naloxone-precipitated jumping) in morphine-dependent mice [8]. The different fractions isolated from *S. leriifolia* leaf, have appreciable antioxidant effects. Whole methanolic extract, precipitates of methanolic extract and most separated fractions showed more antioxidative activity than  $\alpha$ -tocopherol [9]. The leaf essential oil of the plant was characterized by higher amount of  $\beta$ -pinene (31.5%), 1,8-cineole (24.7%) and  $\alpha$ -pinene (17.5 %) [4].

The early onset of vegetative and reproductive growth and also drought and cold tolerance of *S. leriifolia* indicated that this plant could be adopted, considering its medicinal and oil qualities, especially in arid and cold zones. On the other hand, *S. leriifolia* is an endangered species, facing a very high risk of extinction; so it seems that sustainable exploitation along with domestication are important viable ways of conserving *S. leriifolia* habitats.

Germination is a critical stage in the life cycle of plants, hence it plays a key role in crop production. Temperature has significant effects on the onset, potential and rate of germination, and is always the most critical factor determining success or failure of plant establishment [10]. Moisture and temperature are the important factors, determining seed vigour, germination rate and high seedling establishment [11]. Uncertainty of germination (U) is an adaptation of the Shannon index associated with the distribution of the relative frequency of germination. Low values of U indicated that germination more concentrated in time [12, 13].

Previous studies have shown that germination rate usually increases linearly with increasing temperature, at least within a well-defined range, declining sharply in higher temperatures. This temperature range has been defined as cardinal temperatures, i.e., minimum or base temperature ( $T_b$ ), maximum or ceiling temperature ( $T_c$ ) and optimum temperature ( $T_o$ ). Understanding of germination response of seeds to temperature and estimation of cardinal temperatures are useful in screening crop tolerance to either low or high temperature, identifying geographical areas and successfully establish and develop management models [14-16].

Several mathematical models were presented for the description of accumulative germination pattern in response to temperature [17-19]. In general, an inverse linear relation between temperature and times needed to reach a given proportion of germination has been reported [19]. In some reports germination rate of Maguire versus temperatures were used for the calculation of cardinal temperatures [20].

Intersected Lines model (ISL), is one of the nonlinear regression models which have been used in many literatures [18-22]. This model consists of two regression lines that describe the response of germination rate to temperature at sub-optimal and supra-optimal temperatures. The intersection point of this regression lines with X axis was considered as  $T_b$ ,  $T_c$  temperature, and  $T_o$  is the intersection point of two linear regression lines [17]. Quadratic Polynomial (QPN) and Five-Parameters Beta (FPB) models are also two other non-linear regression models which are used in some experiments [23-25]. These models are especially useful for comparing germination rate response to temperature, and model coefficients that can be correlated to physiological processes [15].

Little information is available concerning the germination responses of *S. leriifolia* to temperature, and probably, there is no record of its cardinal temperatures. Whereas domestication of plant species is required to understand the growth requirements, especially that of seed germination, therefore, the purpose of this study were to investigate the effect of different temperatures on germination traits and to calculate the base, optimum and ceiling temperatures of *S. leriifolia* seed germination.

## MATERIALS AND METHODS

This study was conducted at Genetic and Physiology Laboratory of Khorasan-e-Razavi Agricultural and Natural Resources Research Center (KANRRC), Mashhad, Iran, in 2012. The mature seeds of *S. leriifolia* Benth were collected from Bajestan ( $34^{\circ} 28' 70''$  N,  $58^{\circ} 72' 58''$  E) in May 2012.

Because of non-deep physiological dormancy (mechanical dormancy), seed coats were removed. For germination test, 90 mm Petri dishes containing two disks of Whatman No.1 filter papers were used. Seeds were incubated between

two layers of filter papers and germinated at constant temperatures ranging from 0 to 35°C, at 5°C intervals (Binder GmbH, Germany) for 28 days. During the experiment, the germination papers were wetted periodically with distilled water when required. A seed was considered to be germinated when the length of its radicle was about 2 mm. The germinated seeds were counted and removed at 24-hour intervals, continuing till 100% germination was achieved or germinated seeds became stable in three consecutive records. Collected data were used to plot mean cumulative germination percentage curves against time with Three-Parameters Logistic model (TPL) and then the time to 25, 50 and 75% germination ( $D_{25}$ ,  $D_{50}$ ,  $D_{75}$ ) was determined (equations 1, 2) [17].

$$y = \frac{a}{1 + be^{-cx}} \quad (1)$$

$$y = \text{Ln} \frac{\frac{a-y}{y \cdot b}}{-c} \quad (2)$$

where:

$y$ : Germination Percentage (GP) in each time,

$a$ : maximum germination,

$e$ : the base of the natural logarithm,

$x$ : the time to each GP,

$b, c$ : constant regression coefficient.

Germination Percentage (GP), Mean Germination Time (MGT) [26], Germination Rate of Maguire ( $GR_m$ ) [27] and uncertainty of germination ( $U$ ) [13] were calculated using following formulas (equations 3–6):

$$GP = \frac{\sum_{i=1}^k n_i}{N} \quad (3)$$

$$MGT = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i} \quad (4)$$

$$GR_1 = \sum_{i=1}^k \frac{n_i}{t_i} \quad (5)$$

$$U = \sum_{i=1}^k f_i \log_2 f_i, \quad \text{being } f_i = \frac{n_i}{\sum_{i=1}^k n_i} \quad (6)$$

where:

N: number of total seeds,

$n_i$ : number of seeds newly germinated at time  $t_i$ ,

k: last time (day) of experiment,

$t_i$ : time (day) from the beginning of the germination test observation.

The inversion of time required to 50% germination ( $GR_{50=1/D_{50}}$ ) was determined as an index of germination rate [16, 28]. To quantify the response of germination rate ( $GR_m$  and  $GR_{50}$ ) to temperature and determine cardinal temperature for germination, the following models were used.

### I. Intersected-Line model (ISL):

$$f = \text{if } (T < T_o, \quad \text{Region 1 (T), Region 2 (T)}) \quad (7)$$

$$\text{Region 1 (T) = } b(T - T_b) \quad (8)$$

$$\text{Region 2 (T) = } c(T_c - T) \quad (9)$$

### II. Quadratic Polynomial model (QPN):

$$f = a + bT + cT^2 \quad (10)$$

$$T_o = b + 2cT \quad (11)$$

### III. Five Parameters Beta model (FPB):

$$f = \exp(\mu)(T_c - T_b)^\alpha (T_c - T)^\beta \quad (12)$$

$$T_o = (\alpha \cdot T_c) + (\beta \cdot T_b) / (\alpha + \beta) \quad (13)$$

where:

f: germination rate ( $GR_m$  or  $GR_{50}$ ),

T: mean temperature ( $^{\circ}\text{C}$ ),

$T_b$ ,  $T_c$ ,  $T_o$  are base, ceiling and optimum temperatures ( $^{\circ}\text{C}$ ), respectively,

a, b, c,  $\mu$ ,  $\alpha$ ,  $\beta$  are constant regression coefficients.

$T_o$  in QPN and FPB models (Equations 11 and 13) is the first derivative of germination rate. The models were fitted, using the nonlinear regression procedure of SigmaStat 1.0 [29]. Germination data were Arcsin transformed before statistical analysis. Data were analysed using SAS 9.1.3 [30] statistical package. A Completely Randomized Design (CRD) was carried out in eight replications (8x25 seeds at each temperature) and the means were compared by Duncan's multiple range test.

## RESULTS

The temperature significantly ( $p < 0.001$ ) affected all germination traits of *S. leriifolia* (tab. 1). In response to tested temperatures, the highest Germination Percentage (GP) (92.8%) occurred at 15°C, but GP in the range of 10-25°C was not significant ( $p \leq 0.05$ ) (tab. 2). Germination stopped at below 5°C and at above 30°C.

Mean Germination Time (MGT) was significantly ( $p \leq 0.05$ ) decreased with increasing temperature up to 20°C and increased thereafter. The lowest MGT (2.7 day) was observed at 20°C that was not significantly different from that of 25°C (tab. 2).

Table 1.

Mean squares from analysis of variance (ANOVA) for seed germination traits of *Salvia leriifolia* Benth.

Source of variation	df	GP	MGT	GR <sub>m</sub>	U	df	GR <sub>50</sub>	Df	D <sub>25</sub>	df	D <sub>50</sub>	df	D <sub>75</sub>
Temperature	7	13777	313	76.3	4.96	6	0.31	5	328	4	395	4	158
Error	56	56.4	0.23	0.61	0.11	47	0.003	40	0.21	33	0.46	28	1.50
R <sup>2</sup> (%)		0.96	0.99	0.94	0.85		0.93		0.99		0.99		0.94

As shown in table 2, by increasing temperature up to 20°C, Germination Rate (GR<sub>m</sub> and GR<sub>50</sub>) was increased. As temperature increased beyond 20°C, GR<sub>m</sub> and GR<sub>50</sub> were significantly decreased. Figure 1 showed that cumulative GP was different at various temperatures. The highest GP (79.3%) occurred during the 72 hours after the start of test at 20°C. Analysis of variance showed that the time required for 25, 50 and 75% (D<sub>25</sub>, D<sub>50</sub>, D<sub>75</sub>) germination was significantly ( $p \leq 0.001$ ) different among treatments (tab. 1). The lowest D<sub>25</sub>, D<sub>50</sub>, D<sub>75</sub> were 1.7, 2.2 and 2.9 day at 20°C, respectively (tab. 2, fig. 1).

Table 2.

Means of Germination Percentage (GP), Mean Germination Time (MGT), Germination Rate of Maguire (GR<sub>m</sub>), Germination Rate of 1/D<sub>50</sub> (GR<sub>50</sub>), Uncertainty of the germination (U) and times to 25, 50 and 75% Germination (D<sub>25</sub>, D<sub>50</sub>, D<sub>75</sub>) of *Salvia leriifolia* seeds at different temperatures

Temperature (°C)	GP (%)	MGT (days)	GR <sub>m</sub> (seed/d)	U (bit)	GR <sub>50</sub> (1/D <sub>50</sub> )	D <sub>25</sub> (days)	D <sub>50</sub> (days)	D <sub>75</sub> (days)
0	0.0 <sup>d</sup>	–	–	–	–	–	–	–
5	61.0 <sup>b</sup>	19.5 <sup>a</sup>	0.72 <sup>de</sup>	1.82 <sup>ab</sup>	0.05 <sup>d</sup>	18.3 <sup>a</sup>	20.6 <sup>a</sup>	28.5 <sup>a</sup>
10	92.5 <sup>a</sup>	5.7 <sup>b</sup>	3.59 <sup>c</sup>	2.06 <sup>a</sup>	0.19 <sup>c</sup>	4.1 <sup>b</sup>	5.3 <sup>b</sup>	6.4 <sup>b</sup>
15	92.8 <sup>a</sup>	3.5 <sup>c</sup>	6.49 <sup>ab</sup>	1.60 <sup>bc</sup>	0.36 <sup>b</sup>	2.4 <sup>c</sup>	3.0 <sup>c</sup>	3.6 <sup>c</sup>
20	89.8 <sup>a</sup>	2.7 <sup>d</sup>	7.22 <sup>a</sup>	1.33 <sup>c</sup>	0.46 <sup>a</sup>	1.7 <sup>d</sup>	2.2 <sup>d</sup>	2.9 <sup>c</sup>
25	86.9 <sup>a</sup>	3.0 <sup>d</sup>	6.32 <sup>b</sup>	1.57 <sup>bc</sup>	0.41 <sup>b</sup>	1.9 <sup>d</sup>	2.5 <sup>cd</sup>	3.8 <sup>c</sup>
30	24.8 <sup>c</sup>	4.0 <sup>c</sup>	1.39 <sup>d</sup>	1.37 <sup>c</sup>	–	4.0 <sup>b</sup>	–	–
35	0.0 <sup>d</sup>	–	–	–	–	–	–	–

Values followed by different letters are significantly different at  $p \leq 0.05$  level according Duncan's multiple range test

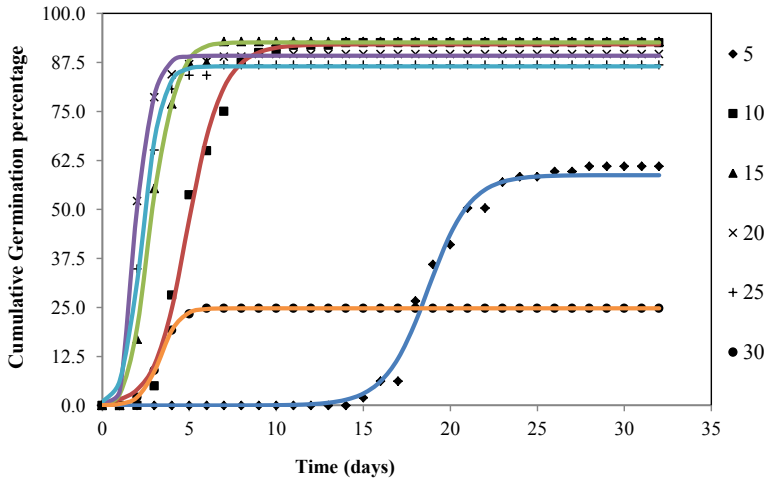


Figure 1.

Cumulative Germination Percentage of *Salvia leiriifolia* Benth. against time for different temperatures

Results also showed that the most uncertainty (U) occurred in low temperatures (5-10°C), but it was significantly decreased with increasing temperature. There was no significant difference for U index between 15 to 30°C.

Calculated cardinal temperatures ( $T_b$ ,  $T_o$  and  $T_c$ ),  $R^2$  and fitted Standard Error (SE) are presented in table 3. All three models were able to reliably predict the rate of germination with  $R^2 > 0.84$ . Figure 2 shows the regression equations of germination rate ( $GR_m$  and  $GR_{50}$ ) versus temperatures for the sub and supra-optimal temperature ranges. Based on these models estimation,  $T_b$ ,  $T_o$  and  $T_c$  were obtained in the range of 1–1.9°C, 18.1–20.8°C and 34.5–38.7°C, respectively. Table 3 also indicated that calculated cardinal temperatures based on  $GR_{50}$ , compared to  $GR_m$  were increased to 0–0.3, 0.7–1.3 and 1.1–2.2 for  $T_b$ ,  $T_o$ ,  $T_c$ , respectively.

Table 3.

Calculated cardinal temperatures of *Salvia leiriifolia* for three fitted models based on Germination Rate of Maguire ( $GR_m$ ) and  $1/D_{50}(GR_{50})$

Germination Rate	Intersected-Line					Quadratic Polynomial					Five Parameters Beta				
	$T_b$	$T_c$	$T_o$	$R^2$	SE	$T_b$	$T_c$	$T_o$	$R^2$	SE	$T_b$	$T_c$	$T_o$	$R^2$	SE
$GR_m$	1.1	34.5	20.1	0.94	1.10	1.6	34.6	18.1	0.84	1.7	1.0	36.5	19.0	0.98	0.66
$GR_{50}$	1.4	35.6	20.8	0.97	0.07	1.9	34.8	18.3	0.85	0.1	1.0	38.7	20.3	0.98	0.05

$T_b$ ,  $T_o$  and  $T_c$  are base, optimum and ceiling temperatures, respectively,  $R^2$  is determination coefficient, SE is a standard error

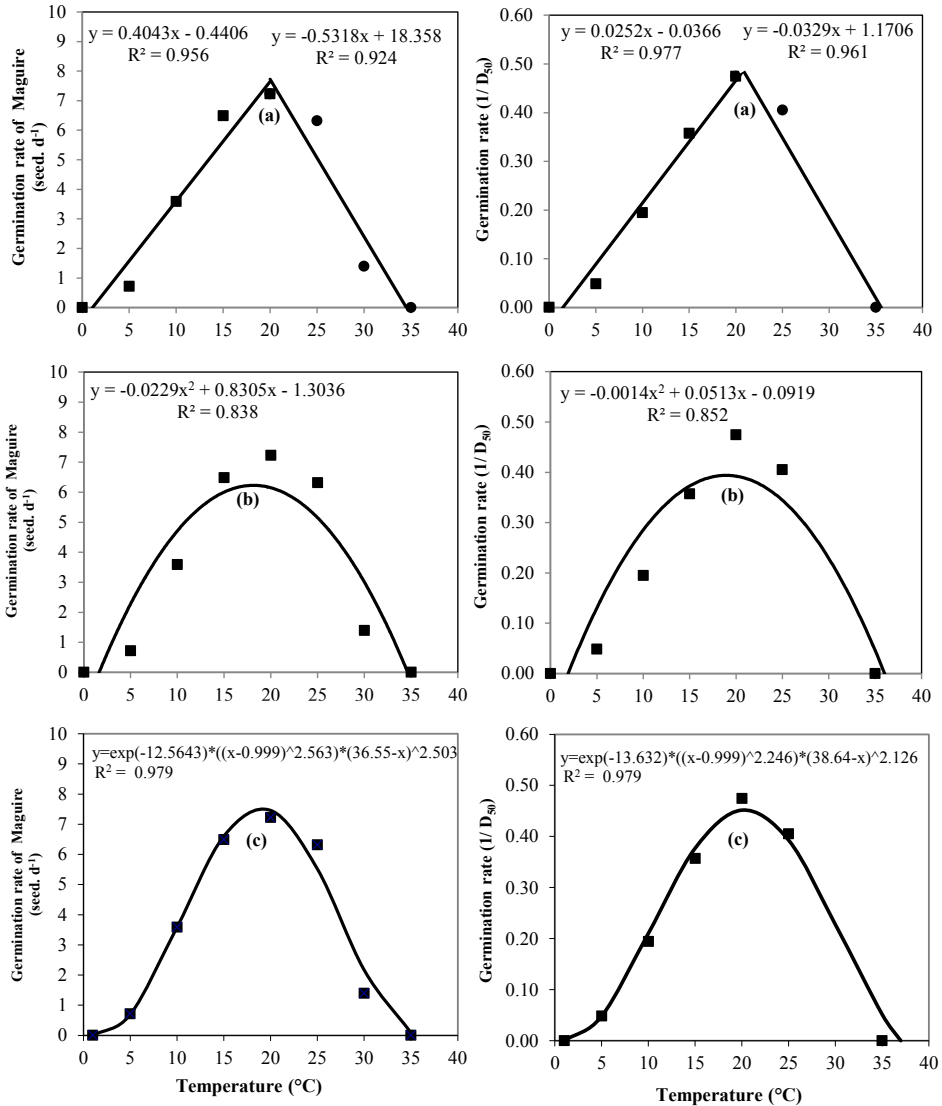


Figure 2.

Effect of different constant temperatures on germination rate of *Salvia lerifolia* Benth. based on three fitted models (a) Intersected-Line (b) Quadratic Polynomial (c) Five Parameters Beta

Five-Parameters Beta (FPB) and Intersected-Line (ISL) models showed better estimation for cardinal temperatures. However, because of the lowest fluctuations in mean residual error for GR (fig. 3) and higher R<sup>2</sup>, the best and more realistic estimation of cardinal temperatures was obtained by FPB model.



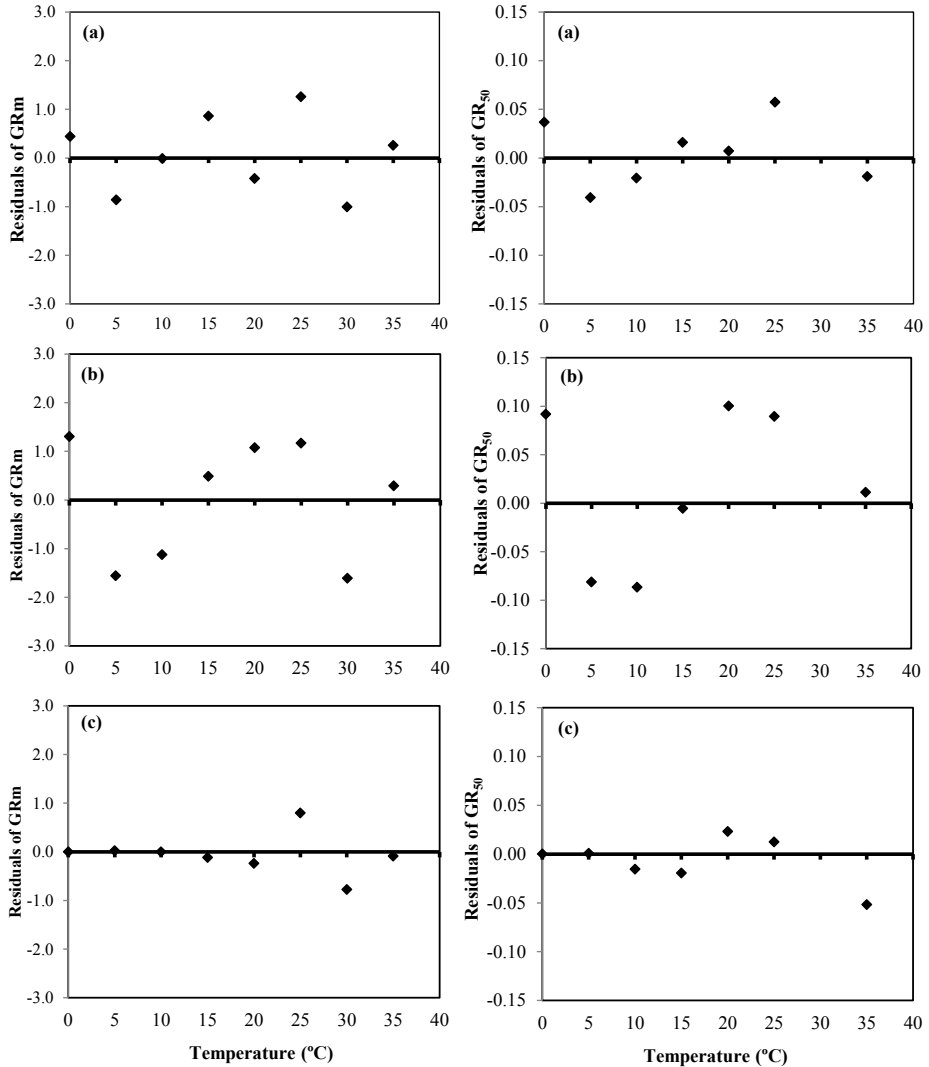


Figure 3.

Mean (symbols) of residual-models error in Germination Rate of Maguire ( $GR_m$ ) and  $1/D_{50}$  ( $GR_{50}$ ) as a function of temperature and three fitted models, Intersected-Line (a) Quadratic Polynomial (b) and Five Parameters Beta (c) for *Salvia leiriifolia* Benth.

## DISCUSSION

Results indicated that *Salvia leiriifolia* seed germination was inhibited by temperatures lower than 5°C and higher than 30°C. Serry et al. [31] also reported no seed germination in 4°C for *S. leiriifolia* coatless seeds, but seed treatment at 4°C

for one week and then transferring to 25°C had the most effect on germination percentage. Several recent reports have presented the highest GP occurring in the range of 20–30°C for *Nepeta binaludensis* and *N. crassifolia*, 15–20°C for *Zataria multiflora*, 20–25°C for *N. glomerulosa*, 25–30°C for *Dysphania botrys*, 15–30°C for *Thymus kotschyanus*, *Rubia tinctorum* and *Achillea millefolium* ssp. *elburensis* [32] and also 15–25°C for Dill, Fennel and Ajowan [20]. Jami Al-Ahmadi and Kafi [19] showed that *Kochia scoparia* seed germination occurred over a wide range of temperatures, from 3.5 to 50°C.

Results also showed that the lowest MGT occurred at 20°C. Some reports showed that MGT could be highly indicative for emergence performance in seed lots of pepper [33] and maize [26, 34]. Germination rate is another aspect of seed germination affected by temperature. Adam et al. [16] showed that GR differed among species and seed lots within species. Temperatures higher than 25°C led to significantly ( $p \leq 0.05$ ) reduced GP and GR. Most reports showed that temperature increased up to optimum followed by increased GR, but declined afterwards [11, 22, 32, 35]. Hardegee [15] reported that there was a large error in predictions of seedling emergence in early spring due to seed degradation and lowering GR at high temperatures. Based on these findings, *Salvia leriifolia* had non-uniform germination, especially in low temperatures. Rojas-Arechiaga et al. [12] also reported that seeds of conventional crops have rapid and uniform germination, while in wild plants, because of seed dormancy, germination is delayed and it is more non-uniform.

Results indicated that the general trend of germination rate based on  $D_{50}$  depicted a reduction in  $GR_{50}$  with temperatures higher or lower than 20°C (tab. 2). This observation is consistent with previous work on pea [28]. In the former study, the response of temperature to  $D_{50}$  followed as a U shape curve, that is typical for many developmental stages, including germination. Our finding also showed that Bajestan ecotype did not reach 50% germination ( $D_{50}$ ) at temperatures higher than 25°C, but this trend was the same (U shaped) for  $D_{25}$ .

The results confirmed that optimal temperature range of seed germination was 10–25°C and did not support Hadad Khodaparast and Hosseini [36] findings, who reported that seed germination reached to maximum GP at 4–8°C and GR decreased at a temperature higher than 12°C. Thanos and Doussi [37] reported that seeds of *Salvia pomifera* ssp. *pomifera* and *Salvia fruticosa* have optimal temperature range of 10–20°C. Côme [38] found that seeds of *Salvia officinalis* germinated satisfactorily within the range of 10–25°C, although *S. sclarea* had a broader range of optimal temperatures from 10 to 30°C. Hornok [39] reported that the optimum temperature for seed germination of *Salvia officinalis* was 25°C. Tabrizi et al. [22] reported that cardinal temperatures were 4.4, 19.0 and 25.5°C for *Plantago ovata* and 9.4, 28.8 and 35.0°C for *P. psyllium*. In another experiment, Tabrizi et al. [24] indicated that, based on the regression between germination rate and temperature in seed lots of *Thymus transcaspicus*, the cardinal temperatures ( $T_b$ ,  $T_o$  and  $T_{max}$ ) were 1.2–3.9, 29.1–25.8 and 45–47°C for field seed lot and 1.0–3.3, 24.9–29 and 45–46 for natural seed lot, respectively. For majority of plant species, optimum

and ceiling temperatures have been reported at 15–30°C and 30–40°C, respectively [40]. However, optimum temperature of germination depends on genetic and environmental conditions that the plant evolved [11].

While all three models showed a good predicting ability, Five Parameters Beta (FPB) model had better estimate for cardinal temperatures. Jame and Cutforth [14] have used FPB model for quantifying the relationship between temperature and germination rate. This model was also used for prediction of flowering time in rice [23] and saffron [25] with reasonable results.

## CONCLUSIONS

1. Germination of *Salvia leriifolia* Benth. improved in cold to moderate temperatures (10–25°C), and conversely, the temperatures higher than 25°C strongly inhibited the germination characteristics.
2. Estimating germination rate based on times to 50% germination ( $GR_{50}$ ) had higher determination coefficient ( $R^2$ ) in all three models compare to Maguire method ( $GR_m$ ).
3. The cardinal temperatures calculated by ISL or FPB could be useful guidance for introduction of these species to new areas, in respect to soil temperature to determine proper sowing time, however, more work is needed to clarify this point.

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#### WSKAŹNIKI TEMPERATUR KIELKOWANIA *SALVIA LERIIFOLIA* BENTH.

MAJID DASHTI<sup>1, 2\*</sup>, MOHAMMAD KAFI<sup>1</sup>, HOSSEIN TAVAKKOLI<sup>2</sup>, MAHDI MIRZA<sup>3</sup>

<sup>1</sup>Ferdowsi University of Mashhad  
Department of Agronomy  
Mashhad, Iran

<sup>2</sup>Khorasan-e-Razavi  
Agricultural and Natural Resources Research Center  
Mashhad, Iran

<sup>3</sup>Research Institute of Forest and Rangeland  
Medicinal Plant Research Branch  
Tehran, Iran

\*autor, do którego należy kierować korespondencję:  
e-mail: Majiddashti46@gmail.com

#### Streszczenie

Celem pracy było badanie cech kiełkowania i określenie wskaźników temperatur kiełkowania rośliny leczniczej *Salvia leriifolia* Benth. Badanie laboratoryjne przeprowadzono przy stałych temperaturach z zakresu od 0 do 35°C, w odstępach co 5°C. Badano losowo wybrane diaspory w ośmiu powtórzeniach. W celu opisanie wpływu temperatury na zdolność kiełkowania, zastosowano trzy modele regresji, mianowicie: segmentową regresję liniową (ISL), wielomianu kwadratowego (QPN) i regresję pięcioparametrową (FPB). Najwięcej nasion (92.8%) wykiełkowało w 15°C, przy czym różnice w wartości wskaźników zdolności kiełkowania (GP) w zakresie 10–25°C nie były istotne statystycznie ( $p \geq 0.05$ ).

Proces kiełkowania ustawał w temperaturze poniżej 0°C i powyżej 30°C. Wykazano, że najwyższy wskaźnik szybkości kiełkowania (GR), najkrótszy średni czas kiełkowania (MGT), a także najkrótszy czas kiełkowania 50% diaspor ( $D_{50}$ ) występują w temperaturze 20°C. W temperaturach powyżej 25°C nie uzyskano poziomu 50% kiełkujących nasion. Model FPB najlepiej przybliżał rzeczywiste wartości temperatur. Na podstawie zastosowanych modeli regresji stwierdzono, że temperatury minimalna ( $T_b$ ), optymalna ( $T_o$ ) i maksymalna ( $T_c$ ) wynosiły odpowiednio: 1–1,9°C, 18,1–20,8°C i 34,5–38,7°C.

**Słowa kluczowe:** wskaźniki temperatur kiełkowania, rośliny lecznicze, *Salvia leriifolia* Benth., modele regresji