

Seed Germination and Early Growth Responses of Hyssop, Sweet Basil and Oregano to Temperature Levels

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Abstract

The objectives of this survey were to determine the effect of temperature on germination and seedling growth of Hyssop (*Hyssopus officinalis* L.), Sweet basil (*Ocimum basilicum* L.) and Oregano (*Origanum vulgare* L.) (Lamiaceae family) as well as comparing species regarding germination behavior and growth characteristics. Seeds were germinated on a temperature-gradient bar varying between 5 and 40 °C (with 5 °C intervals). Results indicated that the highest germination percentage of hyssop (92-98%), sweet basil (86-90%) and oregano (74-77%) occurred at 20-30 °C, 25-30 °C and 20-30 °C, respectively; therefore, moderate and warm temperatures are proper for germination of all species. In all species the maximum germination rate obtained at 30 °C. Among all species, Day 10 % of Sweet basil Germination had the lowest value, which indicates faster germination. The cardinal temperatures (base, optimum and ceiling or maximum) were estimated by the segmented model. Base temperature (T_b) was calculated for hyssop, sweet basil and oregano as 3.42, 5.70 and 5.46 °C, respectively. Optimum temperature (T_o) calculated for all species was approximately 30°C, So warmer temperatures are much more proper for them. The species showed different maximum temperatures (T_m) from 42.91 (Oregano) to 48.05 °C (Hyssop). In Hyssop and Sweet basil optimum growth of seedlings were observed at 30°C while Oregano reached its best growth at 25°C. The difference between maximum and minimum temperatures of germination knowing as temperature range (TR) index could show adaptation capability to broad sites for planting and domestication. Regarding this index Hyssop stood in the first place.

Keywords: cardinal temperatures, *Hyssopus officinalis* L., Lamiaceae, medicinal plant, *Ocimum basilicum* L., *Origanum vulgare*

Introduction

Germination necessities are species-specific and the expansion and rate at which the process takes place in non-dormant seeds, is affected by several environmental factors, such as light, oxygen, water and temperature (De Villiers *et al.*, 2002). Temperature range as one of the most significant factors affecting the seed germination process is characterized by cardinal temperatures, i.e., base temperature (T_b) below which it doesn't germinate, ceiling temperature (T_c) above which the germination is zero and optimum temperature (or range of temperatures) (T_o) at which the germination rate is the highest (Phartyal *et al.*, 2003). Generally temperatures below the optimum result in progressively poorer germination (Nykiforuk and Flanagan, 1994) while germination rate is usually increased linearly by temperature, at least within a well-defined range and declines sharply at higher temperatures (Kamkar *et al.*, 2006; Mwale *et al.*, 1994; Ramin, 1997). Temperature affects the onset, potential and rate of germination (Flores and Briones, 2001; Roberts, 1988), and is thereby always the most critical factor determining success or failure of plant establishment (Kader and Jutzi, 2004)

Cardinal temperatures (minimum, optimum and maximum) determine the range of temperatures at which a particular species seeds can germinate. Species dispersion

area, suitable time and place for growing can be estimated by knowing cardinal temperatures. Aromatic plants have wide range of economic uses for many centuries; cooking, medicine, cosmetic, fuel are some of them. Knowing germination procedure will be very useful in growing medicinal crops as this information will help in domestication and successful establishment of the seedlings particularly when there are wild species. Lamiaceae family (Mint Family) is famous for its species richness with medicinal properties, which have been used since early times (Kocabas and Karaman, 2001) as well as many precious species belonging to Papilionaceae family. Little information is known about optimal germination conditions of many labiates while there is a potential commercial interest for them (Estrelles *et al.*, 2010). Since cardinal temperature is very important for seed germination and there is not any comprehensive information about cardinal temperatures and the response of seed germination to varying temperatures for Hyssop (*Hyssopus officinalis* L.), Basil (*Ocimum basilicum* L.) and Oregano (*Origanum vulgare* L.) from Lamiaceae family, the goal of this study was to probe the effects of temperature on seed germination and seedling growth in these medicinal plants and to calculate the base (T_b), optimum (T_o) and ceiling temperature (T_c) for them.

Materials and methods

Source of seeds

Seeds of three species used in study were collected in 2011 from the herbal garden of medicinal plants, Research Station of Faculty of Agriculture, Ferdowsi University of Mashhad located in central part of the Khorasan Razavi province, Iran. No fertilizer was used during plant growth and they were irrigated.

Germination test

Full-grown and complete seeds preferred for germination test were selected after one year of storing in dark condition with temperature just about 25 °C. Firstly sodium hypochlorite (NaOCl) 1% was used to sterilize surfaces of the mature seeds for 5 minutes followed by careful rinsing with pure water. For each experiment, 25 seeds were placed in moistened double-layered Whatman no. 1 filter paper (deionized water was used) in Petri dishes. The Petri dishes were insulated by means of Parafilm to prevent the seeds from drying out and small amounts of water were added as needed. Within a fortnight germination response to temperature was assessed over the range 5–45 °C, at 5 °C intervals using dark germinators with 60–70% relative humidity. Germinated seedlings were counted and removed day by day. A seed was considered as germinated once its radicle stood out through the seed coat at least 5 mm. Counting finished when no seed germinated during 4 successive days.

Total germination percentage and germination rate index were calculated for each species. Total germination at each temperature was calculated from the total number of seeds germinated divided by the total number of seeds. Maguire (1962) method was used to calculate mean germination rate index:

Germination Rate Index = (N_i / D_i) where N_i is the germinated seed number on the i^{th} day and D_i is the number of days from the commencement of the germination test to the i^{th} day. Cumulative curve of germination percent was illustrated in response to the time (day), since the experiment started. Then by using these curves was accounted the experiment start time until receiving the 10 (D10), 50 (D50) and 90% (D90) of germination (Soltani et al., 2002). Seedling shoot length was measured from five seedling 10 days after germination commencement.

Statistical analysis

In order to assess the impacts of different temperature levels on germination behavior of mentioned species analysis of variance (ANOVA) was performed as a standard procedure for complete randomized design. For each species at different temperatures, the data was analyzed. Prior to statistical analysis a normality test was done for all data and percentage data were transformed

$$(\arcsin \sqrt{\frac{x}{100}})$$

when required. The LSD test was used for mean comparisons with $\alpha = 0.05$.

Modeling

Cardinal temperatures were obtained by fitting a segmented model (Soltani et al., 2006) (Equation 1) to data collected for three medicinal species against temperature. Germination rate was calculated as the inverse of the number of days (d^{-1}) to reach 50% germination (D50). Model fitted by using the non-linear regression procedure of Sigma Plot 11.

$$f = a_1 + b_1 T \quad (T < T_{opt}) \quad (1)$$

$$f = a_2 + b_2 T \quad (T > T_{opt})$$

There are four parameters in the bilinear approach, a_1 , b_1 , a_2 and b_2 , from which the cardinal temperatures can be derived.

Difference of maximum and minimum temperature for germination known as Temperature Range (TR) (Equation 2) shows the germination ecological range of species.

$$TR = T_m - T_b$$

In which T_m and T_b indicate temperatures above and below which germination will be stopped.

Results and discussion

Effects on total and germination rate

The effect of temperature was significant ($P < 0.05$) on the total germination percentage of all species (Tab. 1). Almost all the seeds of three species germinated at 10–40 °C under darkness, However, only hyssop seeds germinated (45.33%) at the coolest temperature (5 °C). Probably germination absence of two other species at 5 °C, notes to the short period of 14 days because seed germination reduction at low temperatures is extremely related to germination rate decrease (Finch-Savage and Phelps, 1993). On the other hand, medicinal species usually are wild plants that still have not been domesticated like modified crops and have many undesirable characteristics that must be overcome throughout the time. Some researchers reported that wild plants presented lower germination rate and germination percentage than breeding crops (Evans, 1996; Otero-Arnias et al., 2003; Rojas-Ar chiga et al. 2001).

Results showed that the highest germination percentage of hyssop (92–98.66%), sweet basil (86.66–90.66%) and oregano (74.66–77.33%) occurred at 20–30, 25–30 and 20–30 °C, respectively (Tab. 1). This means that, moderate and warm temperatures are suitable for germination of all species. Total germination percentages for hyssop, sweet basil and oregano did not differ significantly over the range of 20–25, 25–30 and 20–30 °C, respectively. Average germination of hyssop and sweet basil at high temperature (40 °C) shows their ability for germination and likely growth in warmer areas.

Tab. 1. Temperature effects on seed germination percentage (%) and germination rate of Hyssop, Sweet basil and Oregano

Species	Germination Percentage (%)								LSD (p<0.05)
	Temperature (°C)								
	5	10	15	20	25	30	35	40	
Hyssop	45.33 D	81.33 C	88 BC	98.66 A	97.33 A	92 B	85.33 BC	45.33 D	8.82
Sweet basil	-†	30.66 E	74.66 C	82.66 BC	86.66 AB	90.66 A	70.66 CD	56 D	9.54
Oregano	-	14.66 C	62.66 B	74.66 AB	74.66 AB	77.33 A	61.33 B	21.33 C	6.66

Species	Germination Rate (seed/day)								LSD (p<0.05)
	Temperature (°C)								
	5	10	15	20	25	30	35	40	
Hyssop	1.17 F	2.92 E	4.90 D	7.81 C	10.47 B	13.94 A	9.10 BC	4.90 D	1.58
Sweet basil	-†	0.97 E	6.87 D	12.53 B	13.92 B	18.95 A	10.06 C	7.44 D	1.94
Oregano	-	0.40 E	3.9 D	4.97 C	5.80 BC	10.75 A	6.28 B	1.02 E	0.93

Any two means not sharing a letter within row differ significantly (LSD test, P<0.05). †: Dash for each temperature indicates that was not analyzed by ANOVA and mean comparison

Putievsky (1983) reported that germination of sweet basil was not sensitive to temperature, and germinated well over a wide range of temperatures. Results of Thanos *et al.* (1995) indicated that three plants of lamiaceae family including thyme (*Coridothymus capitatus*), savory (*Satureja thymbra*) and oregano (*Origanum vulgare* spp. Hirtum) showed germination percentage to a greater extent at a relatively temperature range, with an optimum around 15-20°C. Furthermore, according to their results oregano had an absolute light requirement in their experiment while, in present survey it germinated well under darkness which is consistent with previous results showing Oregano seeds can germinate equally well in darkness or under white light (Grime *et al.*, 1988).

Estrelles *et al.* (2010) reported that germination of two species of Sideritis (*chamaedryfolia* and *pungens*) from Labiateae family in darkness were noticeably higher than in well lighted condition. Germination rate index (seed/day) can vary from 0 to 100, which higher values represent faster germination rate. Since in present study 25 seeds were used for each replication, this index was between 0 and 25. For all species, the maximum germination rate obtained at 30 °C. However, the highest range of germination rate for hyssop (9.10-13.94), sweet basil (10.06-18.95) and oregano (5.80-10.75) occurred at 25-35, 20-35 and 25-35 °C respectively (Tab. 1). Among these species, sweet basil had greatest germination rate. For three species, the highest germination rate occurred in moderate and warm temperatures like germination percentage, indicating that these temperatures may be optimum range of temperatures for their germination.

Time to D10, D50 and D90% of Germination

Germination rate index is obtained from the sum of the daily germination rate and cannot be used to measure start, middle and final time of all germinated seed doubtless, germination rate reaching D10 (start), D50 (middle) and D90 (final) will be better predictors of the temperature effects on germination rate. Obviously, temperature had a significant effect on D10, D50 and D90 (P< 0.05). As shown in Fig. 1 number of days to reach D50 and D90 of germination changed like a U-shaped curve for all species at tested temperature ranges. However, there was also U-shaped curve for Oregano germination rate (D10). Similarly, D10, D50 and D90 of all species showed a decline by increasing temperature up to 30 °C at which they reached the minimum and subsequently increased thereafter. Olivier and Annandale (1998) stated that U-shaped curve is a typical reaction to temperature for many developmental stages, including germination. Among all species, D10 of Sweet basil had the lowest value, which indicates faster germination. Regarding D10 there was no significant difference in all species within the range of 20 to 40 °C. While for hyssop and oregano D10 values observed at 30 °C had considerable differences compared with other temperature levels. The highest day of D10, D50 and D90 was between 5 and 10°C for all three species. As mentioned above, germination rate decreases at low temperatures result in seed germination reduction. The lowest value of D50 was related to sweet basil as well. Within the range of 15-40°C hyssop and sweet basil showed final germination (D90) in less than 5, while oregano final germination happened in less than 7 days at 40°C. Based on D50 and D90 values, for hyssop, sweet basil and oregano it seems that 25-30°C, 25-30°C and 30°C are optimum temperatures for germination, respectively.

Cardinal temperatures of germination

The cardinal temperatures (base, optimum and ceiling or maximum) were determined by linear regression analysis of the germination rate (1/D50) against temperature. According to the results of the segmented model, cardinal temperatures were different among species (Tab. 2). Base temperature (T_b) was calculated for hyssop, sweet basil and oregano as 3.42, 5.70 and 5.46 °C, respectively. As shown in the current study, hyssop had lowest T_b this means that this species could germinate in lower temperatures rather than two other species. Optimal temperature was calculated for all species approximately 30°C, So warmer temperatures are proper for faster germination of all species. Of course, it is not an unexpected result as these three species are considered as tropical plants. It can be inferred that germination as the first step in life cycle shows ability of plants in other steps of life. It is worth to mention that species showed different maximum temperatures from 42.91 (Oregano) to 48.05°C (Hyssop).

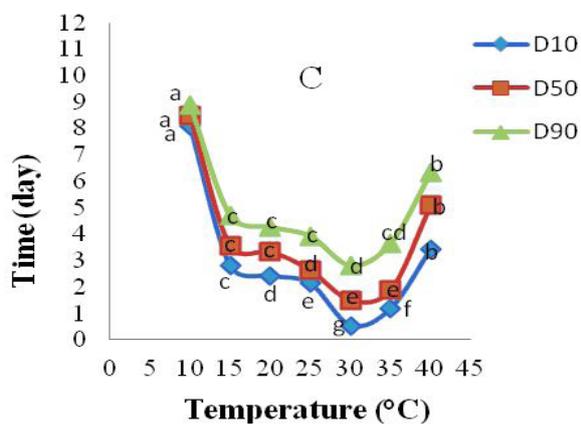
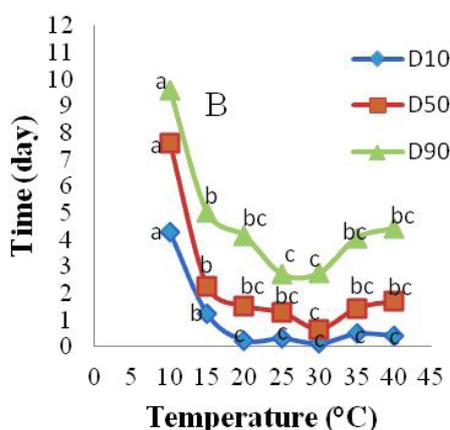
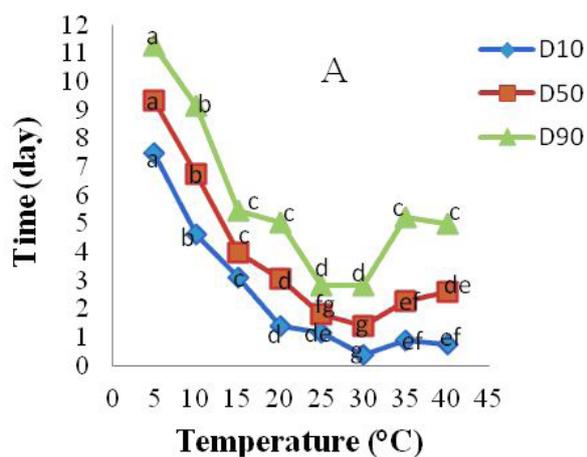


Fig. 1. Comparison of days to D10, D50 (■) and D90% (▲) Of germination for (A) hyssop, (B) sweet basil and (C) oregano Significant at $\alpha = 0.05$

Brändel (2006) reported that the minimum temperature for germination of *Mentha aquatica* was 9 °C and 12 °C in *Lycopus europaeus* and *Stachys palustris* (three species from lamiaceae family), and a maximum temperature of 33 °C for *L. europaeus* and 36 °C for *M. aquatica* and *S. palustris*. For majority of plant species, optimum and ceiling

temperatures have been reported from ranges of 15-30 °C and 30-40 °C, respectively (Copeland and McDonald, 1995). Other studies have shown that the cardinal temperatures for germination depend on species (Ann and Binning, 1987) and genetic and environmental conditions at which plant grows (Iannucci *et al.*, 2000).

Temperature range (TR) index is derived from difference between T_m and T_b . This means that, each species has high TR is able to germinate in wide range of temperatures. According to the result of this experiment hyssop had the highest TR (44.63 °C).

Tab. 2. Cardinal temperatures (°C) of hyssop, sweet basil and oregano on the segmented model

Species	T_b	T^o	T_m	R^2	Temperature Range ($T_m - T_b$)
Hyssop	3.42	30.10	48.05**	0.94	44.63**
Sweet basil	5.70	30.04	46.4	0.98	40.7
Oregano	5.46	30.17	42.91**	0.91	37.45**

T_b , T_o and T_m represent base, optimum and maximum (ceiling) temperatures.

Fig. 2 illustrates that the growth of species seedlings were highly affected by temperature levels. Response of shoot height to various temperature levels was fitted by the third-order polynomial regression for hyssop ($R^2 = 0.94$), oregano ($R^2 = 0.76$) and sweet basil ($R^2 = 0.93$). Totally, within temperature ranges of 15- 40 °C sweet basil seedlings reached the highest height followed by hyssop and oregano, it can be concluded that sweet basil can grow faster and utilize supplies well; therefore, compared with other plants (Intercropping) or weeds it will show better competitive behavior, however, proving this claim needs more experiments in this field. In hyssop and sweet basil optimum growth of seedlings were observed at 30°C while oregano reached its highest growth at 25°C. It seems that besides germination process species growth will also improve by increasing temperature. Chang *et al.* (2005) declared that sweet Basil foliar biomass, dry matter content, and volatile oil content were greatest when temperature remained constant at 25°C compared with constant temperatures of 15 or 30°C. Putievsky (1983) showed that sweet basil germinated well over a wide range of temperatures; however, with increasing temperatures the height of

plants was increased, and the highest dry matter was obtained at 30 °C.

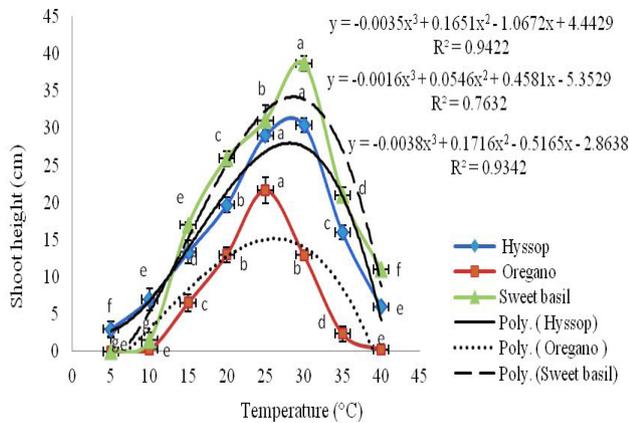


Fig. 2. Effect of temperature on seedlings growth of hyssop (\blacktriangle), sweet basil and oregano (\blacksquare). Fitting the third-order polynomial regression with R^2 of 0.94, 0.76 and 0.93 for hyssop, oregano and sweet basil, respectively. Error bars represent one standard error of mean

Conclusions

Three tested species germinated over a wide temperature range (10-40 °C) under absolute darkness; however, hyssop germinated at 5 °C. Sweet basil and Hyssop showed almost fine germination responses at high temperature (40 °C); furthermore, they demonstrated enhanced seedling growth at higher temperatures, so it can be inferred that higher germination activities at high temperatures could indicate further growth of seedling at warmer temperatures. According to the results of the segmented model, cardinal temperatures including base, optimum and maximum temperatures were various between 3.42-5.70 °C, 30 °C and 42.91- 48.05 °C among species. Jami Al-Ahmadi and Kafi (2007) believed that the ability of seed germination at low temperatures and its rapid germination at high temperatures allows us to use species as a second crop, or to cultivate it twice a year; therefore, maximum utilization of resources can be obtained, especially in arid and semi-arid regions. It seems that the ability of hyssop to germinate at low temperatures and its rapid germination at high temperatures makes it a suitable species approving mentioned hypothesis. Here, we propose temperature range (TR) as an index that could show adaptation capability to broad sites for planting and domestication.

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