



Determination of germination cardinal temperatures in two basil (*Ocimum basilicum* L.) cultivars using non-linear regression models

Hamid-Reza Fallahi^{a,*}, Monavareh Mohammadi^b, Mahsa Aghhavan-Shajari^c,
Fateme Ranjbar^c

^a Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Birjand, Birjand, Iran

^b Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Zabol, Zabol, Iran

^c Department of Agronomy and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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ABSTRACT

Basil has many applications in industrial, cosmetic, pharmacy, foods, perfumery and culinary. Therefore, understanding of its temperature needs is necessary for its appropriate germination, growth and development. In present study three regression models (Intersected-lines model, Quadratic Polynomial model and Five-Parameters Beta model) were used for describing the relationships between germination rate and temperature in green and purple cultivars of basil. Temperature treatments were included of nine constant temperatures including 0, 5, 10, 15, 20, 25, 30, 35 and 40 °C. The experiment was conducted at seed research laboratory of University of Birjand, based on a completely randomized design with four replications. Germination of purple basil started from 10 °C (6%), reached to maximum at 20 °C (73%) and then decreased with increasing temperature until reached to 0% at 40 °C. The highest amount of germination rate was obtained at 25 °C (10 seed day⁻¹), followed by 20 °C treatment. Intersected-lines model was found to be the best model to predict germination rate of purple basil ($R^2 = 0.9$). Based on model output, the amounts of base temperature (T_b), optimum temperature (T_o) and maximum temperature (T_m) for germination of purple basil were 7, 24.97 and 41.84 °C, respectively. Germination of green basil was started from 10 °C (15%), followed by 60–67% in 15–30 °C and finally decreased to 7% in 40 °C. The highest values of germination rate were obtained at 25–30 °C (9.4–9.65 seed day⁻¹). Intersected-lines model showed most suitable fitness in the case of green basil ($R^2 = 0.91$). Based on the regression between germination rate and temperature, the cardinal temperatures (T_b , T_o and T_m) for green basil were 6.11, 28.97 and 43.58 °C, respectively. In addition, temperature range ($T_m - T_b$) for purple and green basil was obtained 34.84 and 37.47 °C, respectively. Totally, the use of the three models confirms the generally known fact that basil needs higher temperatures for germination.

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1. Introduction

Basil (*Ocimum basilicum* L.) as an annual medicinal herb belongs to the Lamiaceae family. It is high value economical and short duration (75–90 days) industrial crop that has a wide range of applications in culinary, cosmetic, food, perfumery and medical industries. Essential oil of the plant is used in flavor and fragrance industries and has several medicinal preparations (Ijaz Hussain

et al., 2008; Kumar, 2012; Singh et al., 2014). Leaves and seeds are the useful parts of the basil (Ekren et al., 2012). This plant is native to southern Asia and is cultivated extensively in countries such as Egypt, France, Greece, Hungary, Indonesia, Iran and Morocco. The main production areas for basil are located in Mediterranean zones and in various regions with temperate and hot climates (Kumar, 2012; Zhou, 2012).

Considering many industrial applications of basil, it is important to determine its climatic needs, especially temperature. Because, temperature determines metabolism and development rates of plants and has considerable effects on the onset, percentage and rate of germination. Therefore, this climatic factor is always the most critical agent in determining the success or failure of plant establishment (Jami Al-Ahmadi and Kafi, 2007). Suitable temperature for seed germination and seedling growth is an important

* Corresponding author at: University of Birjand, Faculty of Agriculture, Agronomy and Plant Breeding Department, Birjand, South Khorasan Province, Iran.

E-mail addresses: Hamidreza.fallahi@birjand.ac.ir (H.-R. Fallahi), mohamadimonavare@yahoo.com (M. Mohammadi), Mahsa.aghavanishajari@stu.um.ac.ir (M. Aghhavan-Shajari), Fateme.ranjbar@stu.um.ac.ir (F. Ranjbar).

factor in determining the best sowing date. Crops can be classified as cool or warm-season based on their temperature needs. Cool-season crops are planted earlier under cooler conditions compared to warm-season crops. Planting in suitable temperature conditions causes the optimum germination, stand establishment and crop performance, while, consequences such as reduced germination, possible frost damage, low and non-uniform stands, poor yield, and replanting associated with too early planting date (Gilbertson et al., 2014). In addition, temperature needs for germination and seedling growth is an important index to determine the suitability of a crop to grow in new areas (Adam et al., 2007).

The main factors affecting seeds germination are soil temperature, water, oxygen, light, chemicals and abiotic regulators. Among all of these agents, temperature is considered as the main factor. It is necessary to identify germination cardinal temperatures including base (T_b), optimum (T_o) and maximum (T_m) temperatures for determine the best planting date of each crop (Parmoon et al., 2015). The amount of germination below base temperature and above maximum temperature is 0%, while optimum temperature is the temperature where germination is maximal (Eberle et al., 2014). Cardinal temperatures for germination in many plants are greatly similar to those of normal vegetative growth (Adam et al., 2007).

Nonlinear regression models have been used to describe germination cardinal temperatures in many crops. Intersected lines model consists of two linear regressions; in the first line germination rate increases up to optimum temperature threshold and in the second line germination rate decreases in response to temperature severe increase. Base and maximum temperatures are derived from the interception of each regression line with the abscissa. Moreover, optimum temperature is the intersection point of two linear regression lines (Jami Al-Ahmadi and Kafi, 2007). Five-parameters beta and quadratic polynomial are two other important models that are used in calculation of germination cardinal temperatures in many herbs (Ranjbar et al., 2013).

Basil is a warm season herb usually propagated by seed, but germination and consequently plant establishment becomes difficult under unfavorable temperature conditions (Zhou, 2012). There is some information in the scientific literatures about the base, optimum and maximum temperatures for basil seed germination. However, less attention has been paid on the use of regression models for calculating of basil thermal needs. In a research on green and purple types of basil, germination and seedling emergence in saline condition were higher at 25 °C than 15 and 35 °C treatments (Ramin, 2006). In another study it has been reported that the highest germination percentage of sweet basil occurred at 25–30 °C. Moreover, optimal temperature for germination of this species was approximately 30 °C, so warmer temperatures are much more proper for basil (Mijani et al., 2013). In similar experiment on Indian basil it was reported that the temperature of 25 °C is optimal for seed germination (Kumar, 2012). In a study about temperature needs for germination of six cultivar of sweet basil was concluded that base temperature of cultivars ranged between 10.1 and 13.3 °C, and the average optimal and maximum temperatures were 35 ± 0 °C, and 43 ± 1.3 °C, respectively (Zhou, 2012).

Because of numerous medical and industrial uses of basil and considering the important role of temperature in its growth, beside low information about thermal needs of this crop especially in initial growth period, the objectives of this research were: (1) effect of different temperatures on germination percentage and rate in basil, (2) calculation of germination cardinal temperature as an indicator of the thermal requirements of subsequent developmental stages, (3) comparison of performance of three different models for calculation of cardinal temperatures and (4) determine whether germination behavior varied between two different cultivars of basil.

2. Materials and methods

In order to determine the germination cardinal temperatures of green and purple cultivars of basil, two separate experiments were conducted in seed sciences laboratory of Sarayan Faculty of Agriculture, University of Birjand during 2014. These experiments were performed using germinators with controlled environment based on completely randomized design with nine constant temperatures (0, 5, 10, 15, 20, 25, 30, 35 and 40 °C) and four replicates of 25 seeds. Basil seeds were germinated in 9 cm diameter petri-dishes with one Whatman No. 1 filter paper moistened with 4 ml of distilled water. Germinated seeds were counted and removed daily for 10 days. Seeds considered to be germinated when the radicles elongated at least 2–3 mm (Fallahi and Khajeh-Hosseini, 2011). At the end of experiments germination percentage, germination rate and mean germination time were calculated using Formula (1)–(3).

$$GP = \frac{n}{N} \times 100 \quad (1)$$

$$RS = \sum_{i=1}^n \frac{S_i}{D_i} \quad (2)$$

$$MGT = \frac{\sum DN}{\sum N} \quad (3)$$

In eq. (1): GP, n and N are germination percentage, germinated seeds and total seeds, respectively. In eq. (2): RS is germination rate, S_i is daily seed germination, D_i is number of day to n computation and n is number of days computation. In Eq. (3): MGT = mean germination time, N = the number of seeds, which were germinated on day D and D = the number of days counted from the beginning of germination (Ranjbar et al., 2013; Fallahi et al., 2013).

For calculation of cardinal temperatures of two basil cultivars the germination rate- temperature relationship was used. X-axis as the independent variable (temperature) and the Y-axis as the dependent variable (germination rate) were considered. In this study three models including Intersected-lines (Eqs. (4) and (5)), Quadratic Polynomial (Eqs. (6) and (7)) and Five-Parameters Beta (Eqs. (8) and (9)) were used (Ranjbar et al., 2013).

$$T_1 = b(T - T_b) \quad \text{if } T \leq T_o \quad (4)$$

$$T_2 = c(T_m - T) \quad \text{if } T \geq T_o \quad (5)$$

$$f = a + bT + cT^2 \quad (6)$$

$$T_o = b + 2cT \quad (7)$$

$$f = \exp(\mu)(T - T_b)\alpha(T_m - T)\beta \quad (8)$$

$$T_o = (\alpha T_m + \beta T_b) / (\alpha + \beta) \quad (9)$$

where, T is temperature treatment; f = germination rate; T_b , T_o , and T_m are base, optimum, and maximum temperatures, respectively; a , b , c , μ , α and β are model parameters.

The quality of each model was evaluated by the values of coefficients of determination (R^2). Germination difference between maximum and base temperature was considered as temperature range ($TR = T_m - T_b$). This index shows the germination ecological range of a plant species. In which T_m and T_b indicate temperatures above and below which germination will be stopped (Mijani et al., 2013).

The statistical analysis was performed by SAS 9.1 and mean comparison was done by duncan multiple range test at the 5% level of probability.

3. Results

3.1. Effect of temperature on germination of purple basil

Effect of different temperatures was significant on germination indices of purple basil (Table 1). Germination occurred in a thermal range between 10 and 35 °C. The first germinated seed was found on the first day at 20, 25, 30, and 35 °C; third day at 15 °C and tenth day at 10 °C thermal treatments. The greatest increase in germination (60%) occurred with increasing temperature from 10 to 15 °C, while its greatest reduction (43%) was observed when temperature increased from 35 to 40 °C. The highest amounts of seed germination percentage in 10 and 15 °C were observed at tenth and fourth days after onset of germination test, respectively. This index was obtained two days after the start of the experiment for 20, 25, 30 and 35 °C treatments. Germination rate showed an increasing trend until 25 °C and then decreased until it reached to zero in 40 °C. The highest value of germination rate and the lowest amount of mean germination time were obtained in 25 °C treatment (Table 2). Overall, thermal range of 20–25 °C provided the best conditions in terms of percentage, rate and time needed for purple basil seed germination.

Germination rate of purple basil had a decreasing trend at temperatures lower and higher than 25 °C (Fig 1). The intersected-lines (ISL) and five-parameters beta (FPB) models were the best models for predicting germination rate of purple basil ($R^2 = 0.9$). Base temperature for germination of purple basil was equal to 7 °C for ISL model and about 5 °C for FPB model. The amount of optimum temperature was around 25 and 28 °C in ISL and FPB models, respectively. In addition, the value of maximum temperature was approximately 41 °C for both models. Quadratic polynomial model showed the lowest coefficient of determination among three tested models. Therefore, its application isn't so useful for determination of purple basil cardinal temperatures. Moreover, temperature range ($T_m - T_b$) of purple basil was about 35 °C based on the mean values obtained from three used models (Table 3).

3.2. Effect of temperature on germination of green basil

Effect of temperature treatments was significant on all germination indices of green basil (Table 1). Germination of green cultivar of basil occurred in the temperature range of 10–40 °C. The first germinated seed was recorded on the first day at 25, 30, and 35 °C; second day at 20 °C; third day at 15 and 40 °C and tenth day at 10 °C treatments. The highest amounts of increase and decrease in germination percentage were observed when temperatures increased from 10 to 15 °C (44%) and 35 to 40 °C (34%), respectively. The highest seed germination percentage was observed on second day at 20, 25, 30 and 35 °C, fourth day at 15 °C, and tenth day at 10 °C. The maximum amounts of germination percentage belonged to thermal range of 15–30 °C. Effects of temperatures higher than 35 °C and lower than 10 °C were significantly preventer on germination percentage of green basil. In addition, the highest amounts of germination rate and the lowest values of mean germination time obtained in 20–30 °C thermal range (Table 2). Totally, green basil had the best germination condition in thermal range of 20–30 °C.

Germination rate of green basil had a favorable condition in thermal range of 25–30 °C (Fig 2). Intersected-lines model (ISL) was the best model for determining of critical temperatures in green basil ($R^2 = 0.91$) (Table 4). Sub and supra linear regression of germination rate showed that the slope for the supra-optimal regression was higher than the sub-optimal regression slope (Fig 2A). Based on estimation of ISL model cardinal temperature of green basil at initial growth period were 6.11, 28.97 and 43.57 °C for T_b , T_o and T_m , respectively. The amounts of coefficient of determination for five-parameters beta model and quadratic polynomial model was

about 8 and 11% lower than ISL, respectively. Therefore, these models have lower accuracy for predicting of cardinal temperatures in green basil. Moreover, the thermal range of seed germination ($T_m - T_b$) for green basil was about 38.8 °C, based on average estimates of three used models (Table 4).

4. Discussion

Recognition of critical temperatures of life cycle stages of a plant is vital to the successful prediction of its maturity, adaptation and yield in a particular location (Yan and Hunt, 1999). Therefore, understanding plant thermal needs through seed germination studies is an effective way to identify suitable areas for its production and consequently for increase of yields (Tolyat et al., 2014). Basil as an important food, cosmetic, perfumery, culinary and medicinal plant is usually propagated by seed, but its germination, seedling growth and finally its establishment in filed condition is occasionally difficult in adverse conditions of temperature (Ramin, 2006).

Seed germination of purple and green basil was influenced by temperature changes. Germination indices of both basil cultivars were low in hot and cold temperatures outside the range of 15–35 °C. This reduced germination could be caused by a decrease in cell metabolism due to low temperature or induced secondary seed dormancy by high temperatures (Cave et al., 2011; Tolyat et al., 2014). Both basil types had favorable germination rate and percentage in about 20–30 °C thermal range (Table 2). Seed germination as one of the most critical periods in the life cycle of plants is influenced by different environmental and genetic factors. When aeration and moisture are not restrictive, both rate and final germination of viable seeds are mostly controlled by temperature (Kamkar et al., 2012). It has been reported that in most plants, a linear increase in germination indices is associated with an increase in temperature from base to optimum temperature, and then more increase in temperature will reduce them to zero (Parmoon et al., 2015). This occurrence was also observed in the present study, where germination indices of two basil cultivars improved by increasing temperature up to 20–25 °C and then showed a decreasing trend (Table 2).

Unlike the results of Mijani et al. (2013) on sweet basil, the amount of final germination of two basil cultivars at 40 and 10 °C treatments was very low. Depending on environment, hormonal conditions and seed dormancy, final emergence and critical temperatures for seed germination may vary. Storage reduces the estimated base temperature, and accordingly these variations may be caused by differences in production history and seed ages used in two compared studies (Zhou, 2012). Moreover, seed germination is influenced by genetic constitution of variety, nutrition of mother plant, seed maturity, seed size and mass, mechanical injury, aging and pathogen factors (Kumar, 2012). Nevertheless, the thermal points of considerable increase (15 °C) and decrease (about 35 °C) in germination percent were similar in current study with the results of Mijani et al. (2013).

Based on intersected-lines model output, optimum temperature for germination of green basil was about 4 °C higher than purple cultivar (Tables 3 and 4). It has been revealed that germination and seedling growth response to temperature can vary by species, and by seed-lots within a species (Adam et al., 2007). In addition, germination thermal range ($T_m - T_b$) for green basil was about 4 °C wider than purple basil (Tables 3 and 4). It seems that this difference is related to environmental and genetic condition. The thermal range between base and maximum temperatures is affected by seed dormancy, often being narrow in dormant seeds and widening as dormancy is lost (Alvarado and Bradford, 2002). As results of Puteh et al., (2010) on rice cultivars revealed that the non-dormant culti-

Table 1

Results of analysis of variance (sum of squares) for effects of different temperatures on some germination indices in purple and green basil.

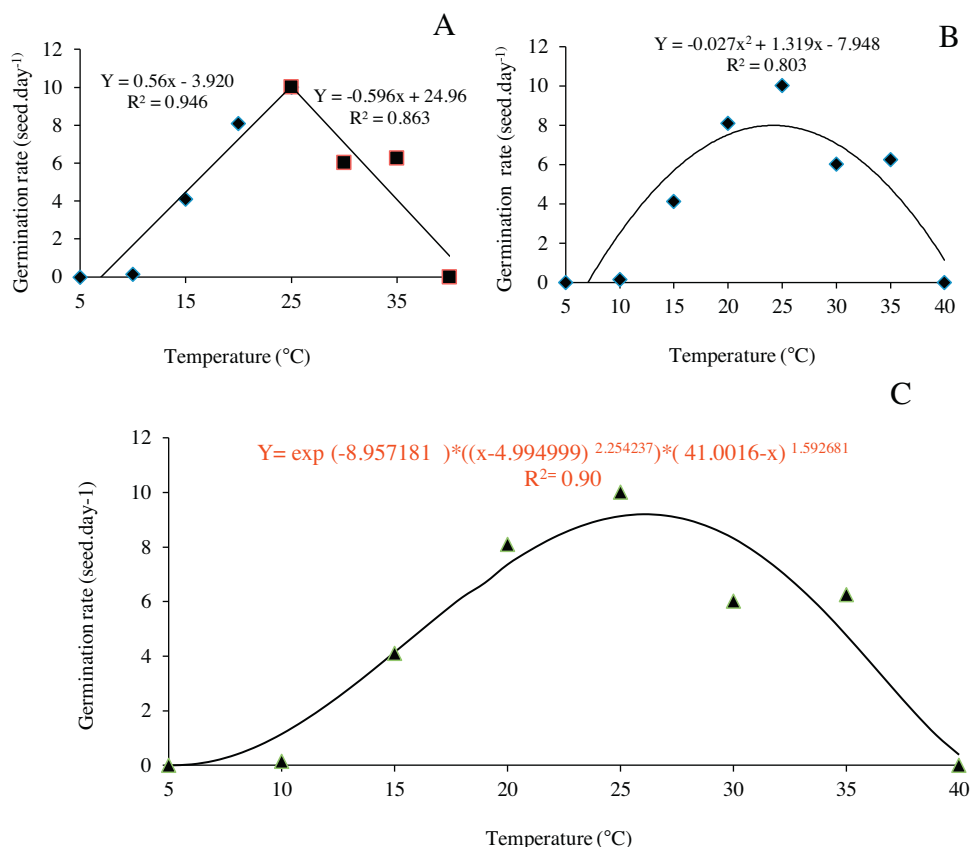
Source of variation	df	Purple basil			Green basil		
		Germination percentage	Germination rate	Mean germination time	Germination percentage	Germination rate	Mean germination time
Temperature	8	33086.2 ^a	499.5 ^a	111.3 ^a	28607.5 ^a	571.6 ^a	168.6 ^a
Error	27	340.0	4.9	0.9	553.0	7.0	4.2
Total	35	33426.2	504.5	112.3	29160.5	578.6	172.8
C.V	–	10.5	11.1	8.8	12.7	11.8	14.8

^a Significant at 5% level of probability based on duncan multiple range test.**Table 2**

Results of means comparison for the effects of different temperatures on some germination indices of purple and green basil.

Temperature (°C)	Purple basil			Green basil		
	Germination percentage	Germination rate (seed. day ⁻¹)	Mean germination time (day)	Germination percentage	Germination rate (seed. day ⁻¹)	Mean germination time (day)
0	00 ^e	0.00 ^e	–	00 ^f	0.00 ^d	0.00 ^e
5	00 ^e	0.00 ^e	–	00 ^f	0.00 ^d	0.00 ^e
10	06 ^d	0.15 ^e	5.00 ^a	15 ^d	0.37 ^d	7.50 ^a
15	69 ^{a,b}	4.11 ^d	4.46 ^b	59 ^b	3.65 ^c	4.20 ^b
20	73 ^a	8.10 ^b	2.46 ^c	67 ^a	7.83 ^b	2.26 ^d
25	66 ^b	10.0 ^a	2.15 ^d	65 ^{a,b}	9.40 ^a	2.21 ^d
30	47 ^c	6.02 ^c	2.62 ^c	66 ^{a,b}	9.65 ^a	2.25 ^d
35	43 ^c	6.25 ^c	2.54 ^c	41 ^c	7.22 ^b	1.79 ^d
40	00 ^e	0.00 ^e	–	07 ^e	0.50 ^d	3.62 ^c

In each column means with similar letter are not significantly different at the 0.05 level of probability.

**Fig. 1.** Effect of different temperatures on germination rate of purple basil based on intersected-lines model (A), quadratic polynomial model (B) and five-parameters beta model (C).

var had lower T_b and T_o compared to the dormant seeds of weedy types.

Model predictions from germination rate in response to temperature changes, had suitable agreement with the observed data

Table 3
Germination cardinal temperatures of purple basil based on three regression models.

Cardinal temperature (°C)	Intersected-lines model	Quadratic polynomial model	Five-parameters beta model
Base	07.00	07.04	04.99
Optimum	24.97	24.15	28.00
Maximum	41.84	41.26	41.00
Temperature range (T_m-T_b)	34.84	34.22	36.01
R^2	0.90	0.80	0.90

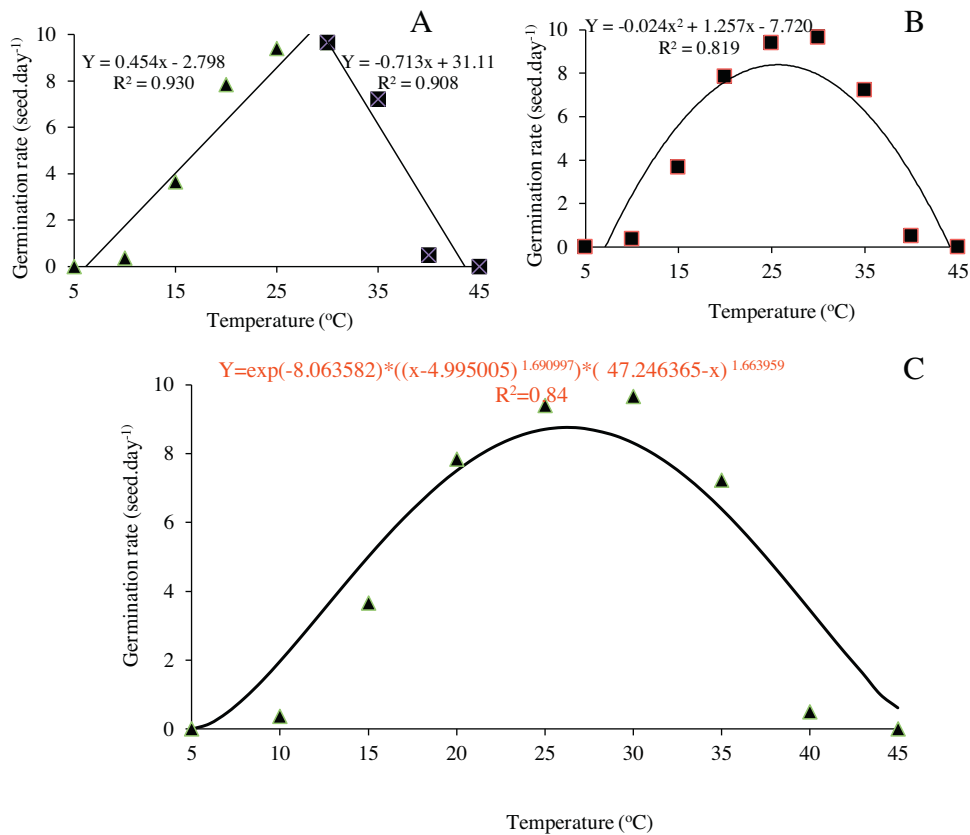


Fig. 2. Effect of different temperatures on germination rate of green basil based on intersected-lines model (A), quadratic polynomial model (B) and five-parameters beta model (C).

Table 4
Germination cardinal temperatures of green basil based on three regression models.

Cardinal temperature (°C)	Intersected-lines model	Quadratic polynomial model	Five-parameters beta model
Base	06.11	07.13	04.99
Optimum	28.97	25.65	26.00
Maximum	43.58	44.17	47.00
Thermal Range (T_m-T_b)	37.47	37.04	42.01
R^2	0.91	0.81	0.84

(Tables 1, 3 and 4 and Figs. 1 and 2). The amount of T_b for two basil types was nearly 5 to 7.1 °C based on prediction of three regression models (Tables 3 and 4). Although the base temperature of basil is relatively low and is able to germinate at temperatures of about 10 °C, however, at low temperatures, germination rate is very low, which creates a problem during field establishment because the seeds can be attacked by a wide range of pathogens before they can germinate and emerge (Zhou, 2012). In addition, the predicted T_0 by three tested models for purple and green basil was in the range of 24.15–28 °C and 25.65–28.97 °C, respectively (Tables 3 and 4). Therefore, both types of basil are nearly warm-season crops and must be planted at relatively high temperatures.

In similar study it has been reported that warmer temperatures are proper for faster germination of sweet basil as the highest germination percent was occurred in 25–30 °C and the amount of T_0 predicted by segmented model was 30 °C (Mijani et al., 2013). Ramin (2006) and Kumar (2012) also concluded that temperature of 25 °C is optimal for seed germination and seedling vigor in basil even under salinity stress condition. In another study on six basil cultivars it was concluded that at temperatures below 20 °C, germination among cultivars was more variable and thus, base temperature of cultivars was different (between 10.1 and 13.3 °C), while their optimal (on average 35 °C) and maximum (on average 43 ± 1.3 °C) temperatures were similar (Zhou, 2012). Despite researches which have been conducted on thermal needs of basil,

application of regression models for calculating of its cardinal temperatures was an innovation in current study. In addition, wider temperature range (0–40 °C) was used in this experiment compared with more previous studies.

5. Conclusion

Germination indices of basil were highest in thermal ranges of 20–25 and 20–30 °C for purple and green cultivars, respectively. Based on prediction of intersected-lines model, the optimum temperatures for germination of purple and green basil were about 25 and 29 °C, respectively. Moreover, base temperature of two studied cultivars was nearly 6–7 °C. Therefore, basil should not be planted before the soil temperature reaches about 7 °C, but its initial growth will be strengthened with delay in planting for reach temperature to desired point (almost 25 °C).

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