Estimating Cardinal Temperatures of Milk Thistle
(Silybum marianum) Seed Germination

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Abstract: The objective of this study was to quantify response of germination rate to temperature and calculating cardinal temperatures in Milk thistle (Silybum marianum). An experiment was conducted at Agricultural Laboratory of Department of Agriculture (Islamic Azad University, Ramhormoz Branch, Ramhormoz, Iran) during 2011. Seeds were incubated on moist filter paper and germinated at constant temperatures ranging from 0 to 45°C (0, 3, 9, 15, 21, 27, 33, 39 and 45°C) in petridishes. A total of four replicates were used in CRD design. Collected data used to plot mean cumulative germination curves against time. To describe the germination rate response to temperature, a segmented model was used. Hence, cardinal temperatures were obtained by fitting a segmented, non-linear regression model to data of germination rate versus temperature. Results showed that the mean of calculated cardinal temperatures were 1.35°C for base, 20.51°C for optimum and 41.81°C for ceiling temperate in Milk thistle. Also, the maximum germination rate at which germination occurs in minimum time, was 2.72 d⁻¹. Calculated cardinal temperatures can be used in other researches with different objects, such as prediction of subsequent development stages of the crops and selection of appropriate sowing date and etc.

Key words: Milk thistle • Cardinal temperatures • Germination • Model

INTRODUCTION

Silybum marianum (L.) Gaertner., Milk thistle, extracts were used as early as the 4th century B.C., became a favored medicine for hepatobiliary diseases in the 16th century and have been used in Europe during this century [1, 2]. Milk thistle grows natively in the Mediterranean [3-4] and is widespread in other regions in the word including Iran [5]. It is currently purported to have value as a liver protectant to lessen damage from potentially hepatotoxic drugs and for treating liver disorders including toxic liver damage caused by chemical, Amanatia phalloides mushroom poisoning, jaundice, chronic inflammatory liver disease, hepatic cirrhosis [6, 7]. The silymarin flavonolignans extracted from Milk thistle seeds protect liver cells against oxidative stress and tissue damage [8-10]. The south-west zone in Iran in which Khuzestan province is located provides suitable conditions for growth of various plants including medicinal plants.

Germination has often been used for evaluating response of development to temperature response functions. Many researchers [11-13] found that a linear relationship resulted when the inverse of time to germination (germination rate) was related to temperature. Temperature is the single most important factor governing the germination [14]. Previous studies have shown that germination rate usually increases linearly with temperature, at least within a well-defined range [15] and declines sharply in higher temperatures [15, 16]. This temperature range has been defined as cardinal temperatures, i.e., a minimum or base temperature (Tb), ceiling temperature (Tc) that germination rate at above of that will be zero and optimum temperature (To) at which the germination rate is highest. Several researchers have shown that the cardinal temperatures for germination depend on species and within species vary significantly [17, 18].

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Nonlinear regression model have been used to describe cardinal temperatures [19-21]. Intersected lines model is one of these methods that have been used in many literatures [19-21]. In this mode Tb and (Tc) are derived from the interception of each regression line with the abscissa and (To) is intersection point of two linear regression lines that describe response of germination rate to temperature [11].

Different types of mathematical models are used to describe the relationship between germination rate and temperature [22]. The using advantage of these functions are that the parameters of these models has a biological concept, Like, cardinal temperatures and the inherent rate of germination and emergence. Therefore, some researchers used these models to obtain Cardinal temperatures [23, 24]. At optimum temperature(s), Germination rate of plants seed is at its maximum and reaches zero at base and ceiling temperatures [24, 25]. Cardinal temperatures may significantly alter between species and varieties [21]. Till now, there is not comprehensive information about cardinal temperatures for Milk thistle and its species. According to the importance of this plant in Iran medicinal industry and adaptation to climatic conditions of Iran, Especially drought tolerance and Recommendation for more cultivation of it, Necessary researches particularly in the fields of seed and plant physiology and evaluating cardinal temperatures for germination of seed seems essential. Therefore, the present study aimed to determine the germination response to temperature and evaluating Cardinal temperatures of milk thistle seeds.

MATERIALS AND METHODS

This study was conducted at laboratory of Department of Agriculture, Islamic Azad University, Ramhormoz Branch, Ramhormoz, Iran, during 2011. Seeds of Milk thistle (Silybum marianum) were obtained from south west of Iran (Ramhormoz, 31°16’N latitude, 49°36’E longitude and 151 m asl). Experiment was performed in germinators. Seeds were incubated on moist filter paper and germinated at constant temperatures ranging from 0 to 45°C (0, 3, 9, 15, 21, 27, 33, 39 and 45°C) in Petri dishes. A total of four replicates were used in CRD design. The germination papers were wetted periodically with distilled water as when required. A seed was considered as germinated if the radicle was visible about 2 mm. Germination was observed at 2 h intervals for the first 24 h and at 10-12 h intervals thereafter for a total of germination period across temperatures. Collected data used to plot mean cumulative germination curves against time. Germination rate, R50, was calculated using the following formula [26]:

\[ R50 = \frac{1}{D50} \]

where, D50 is a Period, which lasts to reach the 50% of germination.

To describe the germination rate response to temperature, the following model was used [22]:

\[ R50 = \frac{f(T)}{G_0} \]

where, G0 is The minimum time for germination at optimum temperatures, Therefore, 1/ G0 indicates maximum germination rate, f(T) is temperature function, which alters between 0 (base temperature) and 1 (optimum temperature) and is obtained from following function (Segmented function):

\[ f(T) = \begin{cases} 
(T-Tb)/(To-Tb) & \text{if } Tb<T<To \\
(Tc-T)/(Tc-To) & \text{if } T<T<Tc \\
0 & \text{if } T>Tc \\
\end{cases} \]

where, T, Tb, To and Tc are mean daily air temperature, base, optimum and ceiling temperatures, respectively. Model parameters were calculated via NLIN procedure in SAS statistical package [27]. Germination percentage was calculated as the proportion of germinated seeds within a replicate. Significance differences within the means of the treatments were calculated using the LSD statistical test [28].

RESULTS AND DISCUSSION

The cumulative germination percentage response against time in different temperatures was varied (Fig. 1). Maximum amounts of germination percentage were at range of 21 and 27°C temperatures and maximum rate of germination rate was obtained at 21°C (Table 1). Tabrizi et al. [29] reported that germination rate was a more sensitive index than germination percentage that can affect on germination seed. Time to start germination, D10, had its maximum amount at 3°C but time to end germination, D90, was obtained at 39°C, so this shows that the seeds began later their germination at temperatures below the optimum temperature while, germination at temperature above optimum temperature...
Table 1: The mean comparison of effect of different temperature on germination percentage and rate, time to 10% of germination (D10) and time to 90% of germination (D90)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Germination percentage</th>
<th>Germination rate</th>
<th>Time to 10% of germination (D10)</th>
<th>Time to 90% of germination (D90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.05</td>
<td>16.00</td>
<td>21.00</td>
</tr>
<tr>
<td>9</td>
<td>88</td>
<td>0.15</td>
<td>5.40</td>
<td>7.16</td>
</tr>
<tr>
<td>15</td>
<td>94</td>
<td>0.26</td>
<td>2.81</td>
<td>4.15</td>
</tr>
<tr>
<td>21</td>
<td>96</td>
<td>0.37</td>
<td>2.00</td>
<td>3.30</td>
</tr>
<tr>
<td>27</td>
<td>82</td>
<td>0.25</td>
<td>3.11</td>
<td>4.40</td>
</tr>
<tr>
<td>33</td>
<td>46</td>
<td>0.12</td>
<td>6.73</td>
<td>8.90</td>
</tr>
<tr>
<td>39</td>
<td>46</td>
<td>0.07</td>
<td>12.24</td>
<td>23.10</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values followed by different letters are significantly different at p<0.01 level according to LSD test.

Table 2: Calculated cardinal temperature and R² of fitted segmented model

<table>
<thead>
<tr>
<th>Tb</th>
<th>To</th>
<th>Tc</th>
<th>G₀</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.35</td>
<td>20.51</td>
<td>41.81</td>
<td>2.72</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Tb, To and Tc are base, optimum, ceiling temperatures, respectively. G₀ is the maximum germination rate or minimum day to germination. R² is correlation coefficient.

Fig. 1: Cumulative germination percentage against time (day) for different constant temperatures

Fig. 2: Fitted segmented model to germination rate against different constant temperatures in Milk thistle.

The results of present study confirmed that in the normal conditions, the germination rate of the species is highly affected by temperature and findings are consistent with other works on different plant species [11-15]. Other studies have shown that the cardinal temperature for germination depend on species and within species vary significantly between genotypes [33].

CONCLUSION

The fitted model for germination rate is given in Figure 2. According to the data distribution, Root Mean Squares Error (RMSE) and R², the segmented model could well predict germination rate. The values of RMSE and R², related to germination rate function, were 0.023 and 0.99, respectively. Thus, segmented model could well describe the relationship between temperature and germination rate. Segmented model parameters i.e. Calculated cardinal temperatures (base, optimum and ceiling) and R² are presented in Table 2. The slope of line showed positive response for temperature between Tb and To and negative response between To and Tc. The values of Tb, To, Tc and G₀ for Milk thistle were 1.34, 20.51, 41.81°C and 2.72 days, respectively. The G₀ value for germination rate was 2.72 d which indicates maximum germination rate was 0.042 h⁻¹ at optimum temperature. Jame and Cutforth [23] have used a Beta function for quantifying the relationship between temperature and germination rate. Based on findings of Evers [30], fast germination probably increases radical emergence from seed and more available water for plant and as well help to better establishment of seedling. Optimum temperature of germination depend on genetic and environmental conditions that plant grows during it [31]. For majority of plant species, optimum and ceiling temperatures have reported from ranges of 15-30°C and 30-40°C, respectively [32].
Segmented model was successful in describing germination response to temperature. So, cardinal temperature of germination can be determined by using this model. Calculated cardinal temperatures can be used in other researches with different objects. For example, the cardinal temperatures could be used for prediction of subsequent development stages of the crops [11-19].

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REFERENCES


