SEEDS GERMINATION PARAMETERS CHANGES OF SUAEDA PASTORAL WILD PLANT UNDER THE EFFECT OF MENTHOL ESSENTIAL OIL CONCENTRATION AT VARIED TEMPERATURES

Mohamed I. ALzarah¹ and Mohammed M. Elgarawany²*

¹Department of Environmental and Natural Resources, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Hassa 31982, Saudi Arabia.
²Research and Training Station, King Faisal University, P.O. Box 420, Al-Hassa 31982, Saudi Arabia.

Abstract

Experiment was to study the effect of menthol concentrations [(0 (C₀), 0.25 (C₁), 0.50 (C₂), 1.00 (C₃), 1.25 (C₄), 1.50 (C₅), 1.75 (C₆) and 2.00 % (C₇)] and varied temperatures [10-20 (T₁), 15-25 (T₂), 20-30°C (T₃)] on the seed germination attributes of the Suaeda wild desert plant. A split plots design with three replicates was used for the layout of the study. Various parameters of seeds germination were monitored within 14 days. Results indicated that except treatments C₆ and C₇, all other treatments including control enhanced seed germination attributes under different temperatures, after 8 - 9 days of the sowing. However, C₆ and C₇ showed inhibiting effects. There was no significant impact of temperature treatment on all parameters such as cumulative germination speed (AGS), mean germination time (MGT), final seed germination percent (FGP), velocity coefficient of germination (CVG), mean daily germination (MDG) and germination rate (GS) while there were significant effects on peak (PV) and germination value (GV). The CVG and MGT decreased with increasing the temperature while no specific trend was found for MDG and GV with temperature treatments, however, other parameters increased with increasing alternating temperature. Significant differences were observed when menthol was applied at different concentrations regarding all seed germination parameters, however, C₀ and C₁ produced the highest values, which were significantly declined at C₆ and C₇. The non-significant effects were found in the interaction between the treatments to FGP, CVG, MDG and GS. AGS, MGT, PV and GV.

Key words: accumulated germination speed; alternating temperatures; final seed germination percentage; mean germination time; menthol; Saudi Arabia; Suaeda.

Introduction

The selection of non-traditional plants that have important environmental benefits and withstand the harsh changing conditions has great importance to achieve agricultural sustainability. These plants are exposed to salt, thermal, dry stresses and show resistance to changing conditions, especially in the desert environment. Saudi Arabia is characterized by arid climate of high temperature and suffers from less natural vegetation, especially pastoral ones. Therefore, farmers working in the culturable regions are trying to provide pastoral plants to re-breeding and propagation. Suaeda is among these plants, which grow in eastern areas and others regions of the kingdom and is known as seep weeds and sea-blitzes. It grows in saline, alkaline and arid soil habitats and needs Na⁺ in their environment, which is saline resistant that can be irrigated with salt water (seawater). Therefore, the importance of this shrub comes from its ability to withstand salinity, high temperatures, and live in arid zone, in addition it is a pastoral shrub which is used for camel and cattle feed. There are about 110 species in genus Suaeda (Entry for Suaeda in Flora of North America). Many species have thick and succulent leaves and are classified as halophile plants. Shaltout et al., (1997) reported that 23% species of coastal lowland in eastern Saudi Arabia where soil pH and EC are high belong to Suaeda vermiculata. Similarly, they also identified the vegetation of Suaeda aegytiaca in Eastern...
province of Saudi Arabia (Shaltout et al., 1996). In another study, they found that Suaeda aegyptiaca plants were growing in high pH and EC soils (Shaltout et al., 1993). Furthermore, Al-Amro et al., (2018) identified the Suaeda vermiculata and Suaeda aegyptiaca as dominate species in the wetland habitats in central region of Saudi Arabia. Similarly, Li et al., (2019) reported that Suaeda glauca and Suaeda salsa species are grown naturally in saline alkali lands of Bohai coast of China.

Germination of seed is the original and most essential phase in the plant life cycle (Grime and Campbell, 1991). Numerous biotic and abiotic influences, such as temperature, salt, sun, water, concentration of oxygen, and alkalinity of the growth media, affect seed germination. For most plants, the rate of seed germination and seedling growth response to temperature has recently been of great interest (Vassilevska-Ivanova and Teckova, 2002; Ghaderi et al., 2008; Koger et al., 2004). Humidity and temperature are the important factors for seed germination, growth and establishment of plants in arid areas (Evans and Etherington, 1990). To evaluate the characteristics of the germination seeds, knowledge of the effects of temperature on seed germinations is important (Jordan and Haferkamp, 1989). Hot air due to sand movement acts as limiting factor for the seed germination in sandy desert (Tobe et al., 2006). Lin and Tang (2005) reported that the growth of many species in the Grassland of Songnen affected by temperature, salinity, and alkalinity. Temperature plays a very important role among these factors that determine the periodicity germination seed and distribution of species (Guan et al., 2009; Milbau et al., 2009). However, germination rate usually rises linearly with temperature (up to optimum temperature), after which the germination rate drops sharply (Alvarado and Bradford, 2002; Kumar, 2012; Ranjbar et al., 2013; Tolyat et al., 2014; Fallahi et al., 2015). Optimum temperatures for seedling emergence of some African leafy vegetables varied from 25 °C and 31 °C, the maximum between 32 °C to 40 °C and minimum between 2 °C and 13 °C (Motsa et al., 2015). While Baskin and Baskin (2001) indicated that, the optimal temperature of good germination of many species is 10-20 °C.

Temperatures between 15 and 45 °C were considered optimal for Ziziphus lotus (shrub) germination; however, the most acceptable temperature was found at 35 °C (Maraghni et al., 2010).

Physiological process of plant such as seeds germination are impacted by allelochemical substances which release from natural aromatic plants or extracted essential oils from some plants (Brito et al., 2010; Ismail et al., 2012; Rassacifar et al., 2013; Dahiya et al., 2017). Additionally, the chemical components such as allopathic compounds that occur in essential plant oils have an effect on seed germination (Rahimi et al., 2013). The ethanol 9.6% and essential oils (200, 400, 600 and 800 ppm with ethanol 9.6%) of wild mint (Mentha longifolia), coriander (Coriandrum sativum L.), fennel (Foeniculum vulgare), green cumin (Cuminum cyminum L.) and caraway (Carum carvi L.) were prevented seed germination of two weeds plants (Lathyrus annuus and Vicia villosa). While the low rates of germination were recorded with ethanol and at low concentration of essential oil (Rahimi et al., 2013). In another study by Maraghni et al., (2010) on Ziziphus lotus they found that the added polyethylene glycol in the growth media increased the moisture stress and inhibited seed ýgermination. Peppermint oil is extracted from the leaves and herbs of the mint plant that contain high amounts of bioactive substances. Menthol is a major component of peppermint oil in addition to flavonoids, glycosides, acids (such as ascorbic acid) and mineral compounds (Czikow and Laptiew, 1983; Mystkowska et al., 2016).

The goal of this article to evaluate the predictive power of Suaeda seed germination at menthol concentration and different temperature within 14 days. To study also their influences on the seed germination rate and various other seed germination related parameters such as accumulated germination speed (AGS), mean germination time (MGT), final seed ýgermination% (FGP), velocity coefficient of germination (CVG), ýmean daily ýgermination (MDG), germination speed (GS), peak value (PV) and ýgermination value (GV) of Suaeda spp.

Materials and Methods

By using sieve, Suaeda seeds were sterilized with 5% of sodium hypochlorite (NaCIO) for 10 minutes, and then it was washed with running distilled water several times. After that, the seeds were immersed in NaCIO solution (10%) for 3 minutes. The seeds were spread and placed on saturated (with menthol concentrations according the treatments) triple layers of filter paper (Whatman No 41 ashless) in sterile Petri dishes. Twenty cleaned seeds were put in each Petri dish per every concentration of menthol (13,5861 Merck at 99% concentration) and control (saturated with distilled water).

The experiment was carried out in the Collage of Agricultural Science and Food and Research and Training Station, King Faisal University, Kingdom of Saudi Arabia. Split plot in randomized complete block design with three replicates were used to study the effect of menthol
Seeds germination parameters changes of Suaeda Pastoral wild plant under the effect of menthol essential oil

4049

concentrations under the various alternating temperatures. In these varied temperature regimes, 16 hours light with higher temperature and 8 hours dark with low temperature conditions was given to the seeds. The main plots were three different temperatures [10-20 (T1), 15-25 (T2), 20-30p C (T3)] and eight menthol concentrations (0, 0.25, 0.50, 1.00, 1.25, 1.50, 1.75 and 2.00%) which were coded by C0, C1, C2, C3, C4, C5, C6 and C7, respectively and were arranged in sub main plots. Through 14 days, the germinated seeds, which have length sprout 2 mm were counted every 24 h. Germination test was ended after 14 days when the germination of seeds stopped. Following traits were calculated:

1. Final Seed germination percentage (FGP, %): The following formula of ISTA (2015) was used to calculate this parameter:

   \[ FGP(\%) = \left( \frac{Ng}{Nt} \right) \times 100 \]

   Where, FGP = final germination percentage; Ng = number of germinated seeds; and Nt = total number of planted seeds.

2. Seedling emergence was scored through the calculation the mean germination time (MGT, day seed−1) which was calculated by formula constructed by Ranal and Santana (2006) as given below

   \[ MGT(\text{day}) = \frac{1}{k} \sum_{i=1}^{k} \left( \frac{Ni \times Ti}{Ni} \right) \]

   Where, MGT = mean germination time; Ni = the number of germinated seeds on day Ti; Ti = time from the start of the experiment to the ith observation (not the accumulated number, but the number correspondent to the ith observation) and k is the last time of germination.

3. Germination speed (GS) was computed as following equation which formulated by Wardle et al., (1991) using the formula showed below:

   \[ GS(\text{seed day}^{-1}) = \frac{N_1}{T_1} + \frac{N_2}{T_2} + \frac{N_3}{T_3} + \ldots + \frac{N_n}{T_n} \]

   Where, N1, N2, N3, · · · , Nn are the number of germinated seeds observed at time (days or hours) T1, T2, T3, · · · , Tn after sowing (the number of seeds that germinated at the specific time).

4. Coefficient of velocity of germination (CVG) was estimated according to the following formula (Bewley and Black, 1994)

   \[ CVG(\% \text{ day}^{-1}) = \frac{1}{k} \sum_{i=1}^{k} \left( \frac{Ni \times Ti}{Ni} \right) \times 100 \]

5. Accumulated germination speed (AGS) it was estimated according Santana and Ranal (2004) as following

   \[ AGS(\% \text{ day}^{-1}) = \frac{N_1}{T_1} + \frac{N_1 + N_2}{T_2} + \frac{N_1 + N_2 + N_3}{T_3} + \ldots + \frac{N_1 + N_2 + N_3 + N_n}{T_n} \]

   Where, N1, N2, N3, · · · , Nn are the number of germinated seeds observed at time (days or hours) T1, T2, T3, · · · , Tn after sowing. (Not accumulated/cumulative number, but the number of seeds that germinated at the specific time).

6. Mean daily germination (MDG, day−1)

   The following formula can be used to calculate mean daily germination (Czabator, 1962)

   \[ MDG = \frac{\text{Total number of germinated seeds}}{\text{Total number of days}} \]

7. Peak Value (PV, day−1)

   Peak value was estimated by the following formula which given by Czabator (1962) for estimated the peak value.

   \[ PV = \frac{\text{Highest seed germinated}}{\text{Number of days for highest seed germination}} \]

8. Germination Value (GV)

   Germination value was competed by the following equation given by Czabator,

   (1962): \[ GV = PV \times MDG \]

   The recorded data were analyzed using ANOVA technique of SAS computer software. The significant differences between treatment means were calculated according to least significant differences (LSD) at the p < 0.05.

Results and Discussion

Response of mean germination percentage (MaGP) of seeds grown under different menthol concentrations and temperatures to varied time interval:

Fig. 1 depicted the changes of MaGP percentage over the period of fourteen days’ time intervals. The MaGP linearly increased with increasing the day numbers that became steady between ninth and tenth day under all menthol concentrations and ýtemperatures. There was
Table 1: Effect of Temperature degree (T, °C) and menthol concentration (C%) on final seed germination percentage (FGP), accumulated germination speed (AGS), mean germination time (MGT), coefficient of germination velocity (CVG%), mean daily germination (MDG), peak value (PV), speed of germination (GS) and germination value (GV).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FGP %</th>
<th>AGS</th>
<th>MGT</th>
<th>CVG</th>
<th>MDG</th>
<th>PV</th>
<th>GS</th>
<th>GV</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_e (10-20°C)</td>
<td>24.4a</td>
<td>3.75a</td>
<td>4.42a</td>
<td>15.25b</td>
<td>0.716a</td>
<td>0.765a</td>
<td>1.33a</td>
<td>1.08ab</td>
</tr>
<tr>
<td>T_e (15-25°C)</td>
<td>25.2a</td>
<td>4.99a</td>
<td>3.52a</td>
<td>12.4a</td>
<td>0.551a</td>
<td>0.874a</td>
<td>1.41a</td>
<td>0.996a</td>
</tr>
<tr>
<td>T_e (20-30°C)</td>
<td>26.4a</td>
<td>5.02a</td>
<td>3.42a</td>
<td>7.68a</td>
<td>0.80a</td>
<td>1.015a</td>
<td>1.58a</td>
<td>1.834a</td>
</tr>
<tr>
<td>LSD_0.05</td>
<td>3.89</td>
<td>1.201</td>
<td>1.114</td>
<td>1.632</td>
<td>0.198</td>
<td>0.161</td>
<td>0.274</td>
<td>0.674</td>
</tr>
</tbody>
</table>

Means in every column in every treatment followed by different letters are significantly different. *** indicate significant at the 5%, 1%, 0.1% levels, respectively and NS means insignificant at level p<0.05. LSD_0.05 least significant difference at 0.05 level of significance.

Effect of Temperature on germination parameters: Data showed in table 1 represented the variance analysis of the seed growth indices response to various treatment combinations i.e. varied temperatures (10-15, 15-20, 20-30°C) and menthol concentrations (C0, C1, C2, C3, C4, C5, C6 and C7) and their interactions. The results revealed that different temperatures significantly (p<0.05) affect MDG, PV and GV parameters; however, there was no significant effect on the other parameters (AGS, MGT, FGP, CVG, GS). These results correspond to the findings of Gairola et al., (2011) who studied the effect of temperatures on seed germination of *Jatropha curcas*. Similarly, Seal et al., (2018) studied the halothermal time model characterized the germination of the halophyte *Suaeda maritima* grown under different salinity and temperature regimes. On the other side, the same table showed that apart from CVG all other parameters such as AGS, MGT, FGP, MDG, PV, GS and GV were significantly (p<0.0001) affected by the application of menthol concentrations. The data in table 1 also indicated that the interaction effect between the factors has significant impact on AGS, MGT, PV whereas it was non-significant regarding other parameters. These results are consistent with Guan et al., (2009) regarding the germination of *Medicago ruthenica* seeds. Similarly, Maraghni et al., (2010) found that the increased of temperature reduced the seed germination of *Ziziphus lotus*.

Effect of temperature on germination parameters: Results of FGP, AGS, MGT, CVG, MDG, PV, GS and GV of *Suaeda* seeds grown under different temperatures were shown in fig. 3. The data showed that there was significant effect of temperature treatments on MDG, PV and GV parameters. On the other side, the temperature has insignificant effect on FGP, AGS, MGT, CVG and GS. The FGP (24.4-26.4), AGS (3.75-26.4),
PV (0.765-1.015), and GS (1.33-1.58) values increased with increasing temperatures, while the opposite response was appeared in the MGT (3.42-4.42) and CVG (7.68-15.25) parameters. There was no apparent trend regarding the effect of varied temperature on MDG and VG as there values varied from 0.551 to 0.800 and from 0.996 to 1.834, respectively. Maraghni et al., (2010) observed that the optimum temperature 35 °C was best for seed germination and any decrease or increase from optimum temperature one-affect seed germination. Whereas Gairola et al., (2011) indicated that the ideal temperature to germinate J. curcas were 30 °C. Similarly, Copeland and McDonald (1995) stated that the seed germination is a complex process where several reactions and individual factors are impacted by the temperature. Pons (1993) reported that the germination of seeds, dormancy state and their changes for some species during season are related to the changes of seasonal temperature directly.

**Effect of menthol concentrations on seed germination parameters:** The results regarding the effects of menthol concentration on FGP, AGS, MGT, CVG, MDG, PV, GS and GV are exhibited in table 1. The
The maximum days of germination (MaGP) and MaGP% under the effect of menthol concentration% and varied temperature (The values in the Figure were calculated from second order equations between mean germination % and days of germination for every menthol concentration under different temperature). The columns and lines represent MaGP and days of maximum germination, respectively.

Results revealed significant negative effect among different menthol treatments ($C_0$, $C_1$, $C_2$, $C_3$, $C_4$, $C_5$, $C_6$ and $C_7$) with regard to all above parameters of seed germination. The presented data depicted that all seed germination parameters decreased with increasing the menthol concentrations between 0 to 1.5% and there was no response regarding these parameters after 1.5% concentration that indicated that the menthol acted as seed germination inhibitor. Regarding FGP parameter, the higher value 54.33% was obtained at $C_0$ (control), while the low value 0.00% was recorded in $C_6$ and $C_7$ (Table 1). Similar results were found in AGS, CVG, PV, GS and GV where the higher values were 11.88%, 26%, 2.20%, 3.61% and 3.48%, respectively at $C_0$, while the lowest values were zero percent at $C_6$ and $C_7$. The menthol concentration $C_1$ produced the highest MGT (4.12) whereas MDG (1.59) was highest at menthol concentration $C_2$, whilst the menthol concentration $C_6$ and $C_7$ had the lowest values. These results indicated that the menthol act as inhibitor for seed germination parameters because it increased moisture stress and osmotic pressure in the germination medium. These results are in covenant with that obtained by Rahimi et al., (2013) who studied the allelopathic effect of certain essential oils on *Lathyrus annuus* and *Vicia villosa* seed germination. They found that different essential oils stoped germination of *Vicia villosa* seed. A slightly increase in the rates of germination were recorded by ethanol and low concentrations of essential oils. In addition, all essential oils and ethanol reduced *Lathyrus annuus* germination in comparison with control. Similar outcome, were reported by Gabriel et al., (2002) and Soares et al., (2000) where menthol oil from wild grass mint adversely affect the seed germination of commercial cultivated species and weeds.

**The interactional effect of varied temperature and menthol concentration on different germination parameters:** The two-way interactional effect of temperature and menthol concentrations on germination parameters such as FGP, AGS, MGT, CVG, MDG, PV, GS and GV was presented in fig. 3. The results revealed that all parameters were decreased from 0.5% of menthol concentration and at all alternating temperatures. Maximum FGP (55%) was recorded at zero menthol concentration at 20-30°C followed by 51% at zero and 0.25% menthol concentration at 10-20°C. Regarding, CVG parameter, the highest value 29.4 % day$^{-1}$ was obtained in $C_0$ at $T_3$ treatment, while the values were decreased with increasing the menthol concentration and become zero from 1.75 and 2% of menthol treatments under all alternating temperatures. Highest weight of AGS was recorded by the interaction of $C_0$ and $T_2$ (14.23% day$^{-1}$) followed by $C_0$ and $T_3$ (12.67% day$^{-1}$). There was no significant difference between these two treatments. The interaction between $C_5$ and $T_1$ recorded the highest MGT (10.4 days) followed by $C_4$ and $T_2$ (6.246 days). The MGT values increased as the menthol concentration increased *i.e.* $C_5$ at 10-20°C (10.4 day), $C_4$ at 15-25°C (6.245 day) and $C_5$ at 20-30°C (7.7 day$^{-1}$), and then the response declined until it $C_6$ concentration. Regarding MDG the data in Fig. 3 revealed that the highest MDG values were $C_2$ at $T_1$ and $T_2$ while the higher values was found at $C_1$ and $T_2$ treatment. The MDG values were decreased to zero at $C_6$ and $C_7$ under all alternating temperatures.
Fig. 3: Effect of alternating temperature (red dashed lines) on different germination parameters (FGP, final germination percentage), AGS (accumulated germination speed), MGT (mean germination time), CVG (coefficient of germination velocity), MDG (mean daily germination), PV (peak value), GS (speed of germination) and GV (germination value). The dotted line represents the trend line of first order equation.

temperatures. The response of PV had similar trend as MDG. The highest PV values were 2.35 day$^{-1}$, 2.72 day$^{-1}$, and 2.366 day$^{-1}$ at C$_1$ at T$_1$, C$_0$ at T$_2$ and C$_0$ at T$_3$, respectively. In general, the PV decreased with increasing the menthol concentrations under all alternating temperatures. The average values of GS of Suaeda seeds as affected by interaction of two factors were presented in fig. 4. The highest average GS values were 3.59, 3.82 and 3.914% day$^{-1}$ at the interaction treatments between C$_1$ × T$_1$, C$_0$ × T$_2$ and C$_0$ × T$_3$, respectively. The values of GS become zero at C6 and C7 under all alternating temperature treatments. The GS parameter decreased with increasing menthol concentrations under all alternating temperatures. Similarly, the interaction between the treatment combinations on the GV of Suaeda seeds shown in Fig. 3 which indicated that the highest value (7.186) at C$_2$ and T$_3$ combination whereas the highest value (3.154) at C$_1$ and T$_1$ treatment. The other higher values i.e. 3.486 were obtained at C$_0$ and T$_2$. The general trend for GV parameter indicated that it was decreased with increasing the menthol concentrations under all alternating temperatures as the other parameters of seed germination behaved apart from MGT. These
results are in covenent with that obtained by Gairola *et al.*, (2011) who studied the effect of temperatures on seed germination of *Jatropha curcas*. Skrzypek *et al.*, (2015) indicated that menthol oil raises the osmotic pressure of the seed germination media, leads to a high pressure on the seeds, causes the seeds to making balance with the external medium, whereas affects the cell compounds, inhibition germination activity and increasing the dormancy time of seed. Our results indicated that the temperatures applied significantly enhanced the seed germination, however, increasing the menthol concentration reduced the seed growth of the Suaeda.
References


Ocimum basilicum species
Mohamed I. Alzarah and Mohammed M. Elgarawany
and to drought, salt, and alkali stresses. Ecological Engineering, 140: 105593.


