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## ORIGINAL ARTICLE

# Factors influencing seed germination of medicinal plant *Salvia aegyptiaca* L. (Lamiaceae)

Mustapha Gorai \*, Hayet Gasmi, Mohamed Neffati

Laboratoire d'Ecologie Pastorale, Institut des Régions Arides, Médenine 4119, Tunisia

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 Temperature

**Abstract** *Salvia aegyptiaca* is a xerophytic perennial herb belongs to the Lamiaceae family commonly used for medicinal purposes. Laboratory experiments were carried out to assess the effects of temperature and salinity on seed germination and recovery responses after transferring to distilled water. Temperatures between 10 and 40 °C seem to be favourable for the germination of this species. Germination was inhibited by either an increase or decrease in temperature from the optimum (30 °C). The highest germination percentages were obtained at 0 mM NaCl; however, the increase of solution osmolalities progressively inhibited seed germination. The germination rate decreased with an increase in salinity for most of tested temperatures, but comparatively higher rates were obtained at 30 °C. Salt stress decreased both the percentage and the rate of germination. An interaction between salinity and temperature yielded no germination at 300 mM NaCl. By experimental transfer to distilled water, *S. aegyptiaca* seeds that were exposed to moderately saline conditions recovered and keep their ability to germinate mostly at low temperatures. At 300 mM NaCl, germination recovery decreased with increasing temperature and it was completely inhibited at 40 °C.

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## 1. Introduction

The genus *Salvia* (sage) belongs to the Lamiaceae family (formerly Labiatae) and includes over 900 species in the world (Kintzios, 2000; Kamatou et al., 2010). In Tunisia, only 10 species are found (Pottier-Alapetite, 1981). *Salvia aegyptiaca* (L.) is an herb very much branched with stiff and almost spinescent branches having retrorse eglandular hairs (Pottier-Alapetite, 1981). It possesses narrow linear-elliptic to oblong-linear leaves, small and numerous flowers and a pale violet corolla. Nutlets black, 2 × 1 mm, mucilaginous on wetting (Siddiqi, 1984). In the world, *S. aegyptiaca* extended from the Canaries to Asia, through northern Africa and the Middle East. In Tunisia, it is rare in the north, quite common in central and

\* Corresponding author. Tel.: +216 75633005; fax: +216 75633006.  
 E-mail address: [gorai.mustapha@yahoo.fr](mailto:gorai.mustapha@yahoo.fr) (M. Gorai).



widespread in the south. It occurs on dry rocky or gypsum soils of the sunny and well drained piedmont, regs, hamadas and deserted rangelands (Pottier-Alapetite, 1981). It is commonly used in local folk medical practices and in cosmetics. For example, the seeds are used as a demulcent for diarrhoea and for piles (Ghazanfar, 1999). The whole plant is used in diarrhoea, gonorrhoea and haemorrhoids, eye diseases, and as an antiseptic, antispasmodic and stomachic (Rizk and El-Ghazaly, 1995). It is also used in cases of nervous disorders, dizziness and trembling (Hussein, 1985). Phytochemically, the whole plant contains flavonoids, tannins, sterols/triterpenes and coumarins (Al-Yahya et al., 1990). Detailed information on germination patterns is important not only for successful cultivation, but also for understanding of species establishment, tolerance to abiotic factors and their dynamics in drylands.

Successful establishment of plants largely depends on successful germination (Gorai and Neffati, 2007). Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Several environmental factors such as temperature, salinity, light, and soil moisture simultaneously influence germination (Ungar, 1995; Huang et al., 2003; El-Keblawy and Al-Rawai, 2005, 2006; Gorai and Neffati, 2007). Salinity and temperature are major factors affecting germination in the saline dry areas. They can interact in determining salinity tolerance during germination (Huang et al., 2003; El-Keblawy and Al-Rawai, 2005; Al-Khateeb, 2006; Gorai and Neffati, 2007; Tlig et al., 2008). Initial establishment of species in salt deserts is related to germination response of seeds to salinity and temperature and early establishment usually determines if a population will survive to maturity (Tobe et al., 2000; Huang et al., 2003; Song et al., 2005). Although higher salinity decreases germination, the detrimental effect of salinity is generally less severe at optimum germination temperature (Al-Khateeb, 2006; Gorai and Neffati, 2007; Tlig et al., 2008).

A great deal of work has been done in the field of optimising the conditions to obtain maximum *Salvia* seed germination. Temperature and light are the main factors investigated and most reports concern both factors (Panagiotopoulos et al., 2000). Nevertheless, little is known about salt tolerance during germination stage. This study was conducted to (i) better understand the seed germination requirements of *S. aegyptiaca* and (ii) evaluate the effects of a wide range of saline solution concentrations, temperature, and their interaction on germination percentage, germination rate and recovery responses. Information from this study provides basic knowledge about germination requirements that can be used for re-establishing projects.

## 2. Materials and methods

### 2.1. Seed collection site

Mature seeds were collected in May 2007, from plants of *S. aegyptiaca* growing on sandy soil in Samaalyate, Ben Guerdan (33°17'N, 10°55'E, at 17 m a.s.l.; South-East Tunisia). This region is arid to semi-arid with a typical Mediterranean climate, characterised by irregular rainfall events and a harsh dry summer period. Annual precipitation is 186 mm and annual mean temperature is 19.4 °C.

### 2.2. Germination experiments

Seeds were surface sterilized in 0.58% sodium hypochlorite solution for 1 min, to avoid fungus attack, washed with distilled water and air-dried before being used in the germination experiments. 90 mm Petri dishes containing two disks of Whatman No. 1 filter papers with 5 ml of test solution were prepared. Germination experiments were conducted, in darkness, in incubators set at 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C (Luminincube II, analys, Belgium; MLR-350, Sanyo, Japan). Seeds were germinated in 0, 50, 100, 200 and 300 mM NaCl solutions under these temperatures. A completely randomised design was used in the germination tests. Four replicates of 25 seeds per treatment were used. During 20 days the germinated seeds were counted and removed every two days. A seed was considered to have germinated when the emerging radicle elongated to 2 mm. Distilled water equal to the mean water loss from dishes was added every two days to maintain salt concentration near the target levels throughout the germination period.

### 2.3. Methods of germination expression

The germination rate was estimated using a modified Timson's index of germination velocity =  $\Sigma G/t$ , where  $G$  is the percentage of germinated seeds at two-day intervals and  $t$  is the total germination period (Khan and Ungar, 1984). The maximum value possible for our data using this index was 50 (i.e., 1000/20). The greater the value, the more rapid is the germination. Seeds which did not germinate after 20 days of NaCl treatments were transferred in distilled water for 20 additional days, under the same conditions, to study their germination recovery. The recovery percentage was determined by the following formula:  $(a - b)/(c - b) \times 100$ , where  $a$  is the total number of germinated seeds after being transferred to distilled water,  $b$  is the total number of germinated seeds in saline solution, and  $c$  is the total number of seeds.

### 2.4. Statistical analysis

Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance (Badger and Ungar, 1989). Data were analysed using SPSS 11.5 (SPSS, 2002). A two-way analysis of variance (ANOVA) was carried out to test effects of the main factors and their interaction on the rate and final percentage of germination. Student–Newman–Keuls test was used to estimate least significant range between means.

## 3. Results

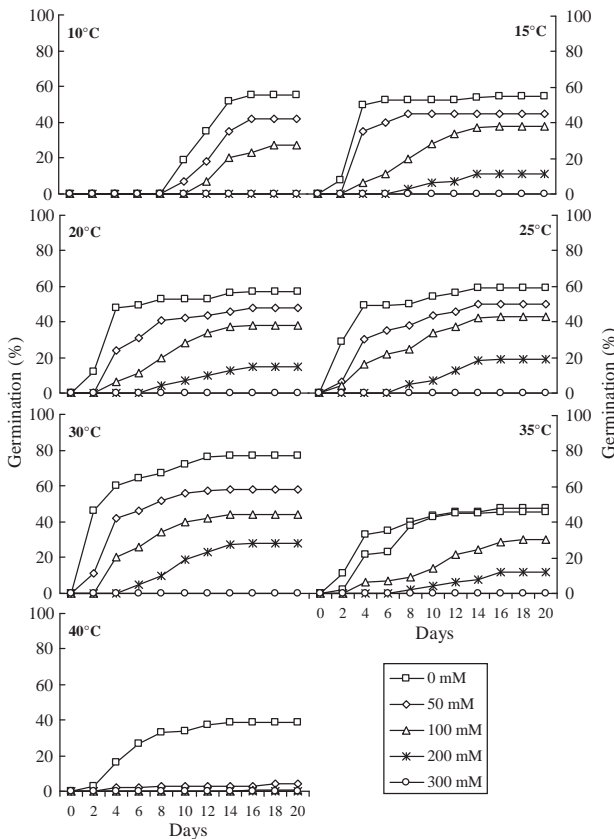
### 3.1. Effects on final germination

Temperature, salinity and their interaction significantly ( $P < 0.0001$ ) affected the final percentage of germination of *S. aegyptiaca* (Table 1). In response to the tested temperatures, the highest germination percentage (77%) of *S. aegyptiaca* occurred at 30 °C and totally declined at 5 and 45 °C (Fig. 1). Germination in distilled water was the highest. However, it decreased significantly with an increase in NaCl concentrations

**Table 1** A two-way ANOVA of the effects of salinity (*S*), temperature (*T*), and their interaction on seed germination characteristics of *Salvia aegyptiaca*.

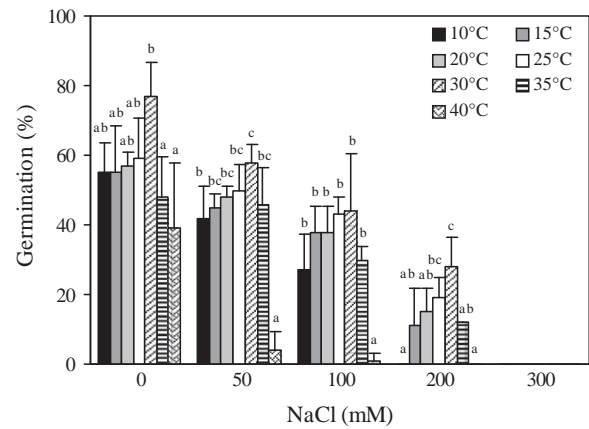
Dependent variable	Main factors		Interaction ( <i>S</i> × <i>T</i> )
	<i>S</i>	<i>T</i>	
Germination percentage	195.70	29.11	3.58
Germination rate	245.83	44.64	5.27
Germination recovery	61.05	25.20	8.95

Note: Data represent *F*-values significant at *P* < 0.0001.

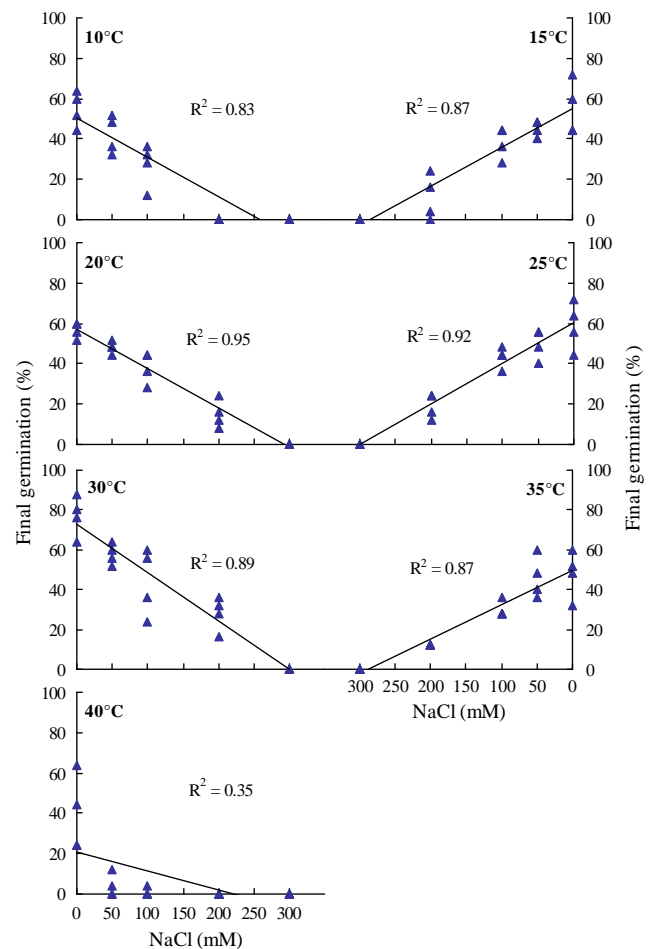


**Figure 1** Cumulative germination percentages of *Salvia aegyptiaca* seeds during 20 days at different temperatures (10, 15, 20, 25, 30, 35 and 40 °C) and NaCl concentrations (0, 50, 100, 200 and 300 mM). At temperature 5 and 45 °C seed germination was completely inhibited (*n* = 4).

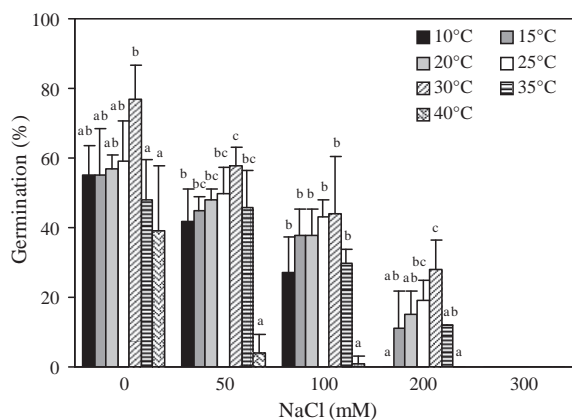
for all tested temperatures (Figs. 1 and 2). Seeds germinated rapidly in distilled water during the initial two days; however, germination was delayed to eight days at 10 °C. The delay of germination increased with increasing NaCl concentrations and was more obvious at 10 °C than other temperatures. The highest germination percentage was found in distilled water followed by 50, 100 and 200 mM NaCl; however, at 300 mM NaCl no germination was recorded (Figs. 1 and 2). There was a strong negative relationship between germination and salinity with a determination coefficient (*R*<sup>2</sup>) ranging from 0.35 to 0.95 (Fig. 3).



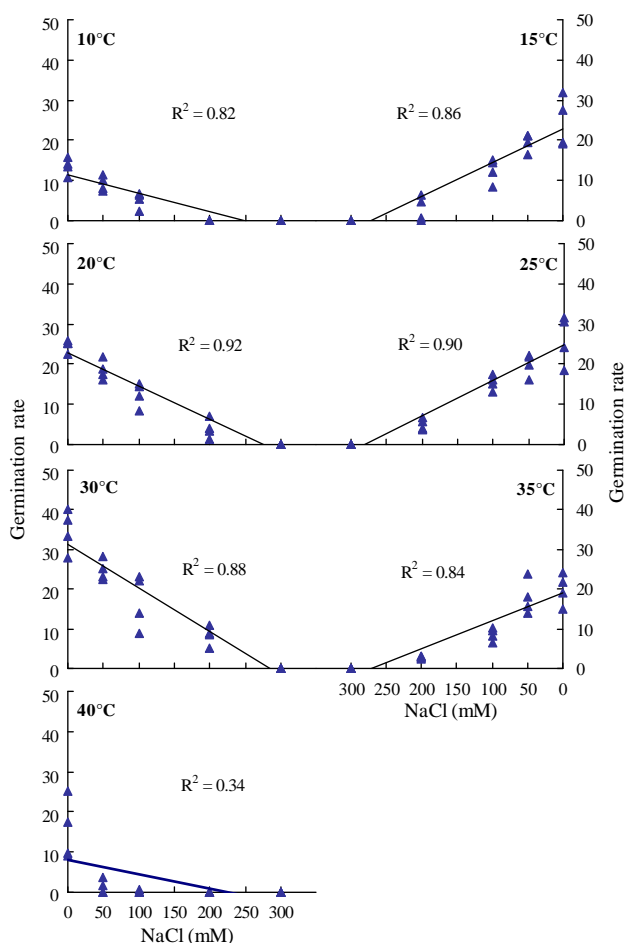
**Figure 2** Mean final germination (±SE, *n* = 4) of *Salvia aegyptiaca* seeds at different NaCl concentrations (0–300 mM) and temperatures (10–40 °C). Values having the same letter are not significantly different at 0.05 significance level (Student–Newman–Keuls’s test).



**Figure 3** Regression plots for mean final germination percentages of *Salvia aegyptiaca* seeds at different NaCl concentrations (0–300 mM) and temperatures (10–40 °C). Values (*n* = 20) are from the five treatments with four replicates.



**Figure 4** Mean rate of germination ( $\pm$ SE,  $n = 4$ ) of *Salvia aegyptiaca* seeds at different NaCl concentrations (0–300 mM) and temperatures (10–40 °C). Values having the same letter are not significantly different at 0.05 significance level (Student–Newman–Keuls’s test).



**Figure 5** Regression plots for mean rate of germination of *Salvia aegyptiaca* seeds at different NaCl concentrations (0–300 mM) and temperatures (10–40 °C). Values ( $n = 20$ ) are from the five treatments with four replicates.

### 3.2. Effects on germination rate

The index of germination velocity calculated using a modified Timson’s index showed that the rate decreased with an increase in salinity (Fig. 4). A two-way ANOVA of the germination rate indicated a significant effect of temperature, salinity and their interaction (Table 1). At 300 mM NaCl, the inhibitory effect of salt on final germination percentage was greater at 40 °C than at 10, 15, 20, 25, 30 and 35 °C. Although NaCl concentrated solutions decrease germination, their effects were generally less severe at the optimal temperature (30 °C). There was a strong negative relationship between rate and salinity with a determination coefficient ( $R^2$ ) ranging from 0.34 to 0.92 (Fig. 5).

### 3.3. Effects on germination recovery

Transfer of seeds to distilled water after salt exposure resulted in an increase in germinated seed number (Table 2). A two-way ANOVA of germination recovery indicated a significant effect of temperature, salinity and their interaction (Table 1). The results show that un-germinated seeds transferred to distilled water recovered except those which were kept at 300 mM NaCl solution at 40 °C (Table 2).

## 4. Discussion

Results from this study indicate that *S. aegyptiaca* seeds can achieve a germination percentage of 77% under non-saline conditions at 30 °C. Germination was inhibited by either an increase or decrease in temperature from this optimal temperature, with an increase of the fraction of moistened and not germinated seeds (Figs 1 and 2). Thanos and Doussi (1995) reported that seeds of two Crete sage species, *S. pomifera* ssp. *pomifera* and *S. fruticosa*, have optimal temperature range of 10–20 °C. In the former study, the highest germination percentages of both species (70–80%) were consistent with those obtained in our experiments (77%). Côme (1993) found that seeds of *S. officinalis* germinate satisfactorily within the range of 10–25 °C, whereas those of *S. sclarea* have a broader range of optimal temperatures between 10 and 30 °C. According to Neffati (1994), the variation in the optimal temperature depends on the considered species, although for the majority of southern Tunisian species germination occurred over a wide range of temperatures and 20 °C appears to enhance their germination. This variation in the optimal temperature and the germination rate between species constitutes some adaptive strategies to harsh environmental conditions. It was shown (Gorai et al., 2006; Gorai and Neffati, 2007) that temperature above the thermal optimum provoked an inhibition of germination and irreversible damage. For example, *S. officinalis* seeds did not germinate at 40 °C which was considered as the temperature limit (Oberczian and Bernath, 1988).

Germination of many species is light independent, and some others present higher germination percentages in darkness than in light (Baskin and Baskin, 1998). In our experiments, it should be noted that petri dishes were removed from dark incubators every two days to check germination; thus, seeds may have received enough light to promote germination. In the former case it is difficult to conclude on light: dark requirement of *S. aegyptiaca*. Working on the same



**Table 2** Recovery (*R*) and total (*T*) germination percentages of *Salvia aegyptiaca* seeds after transfer to distilled water at different temperatures (10–40 °C).

Temperature (°C)	NaCl (mM)							
	50		100		200		300	
	<i>R</i>	<i>T</i>	<i>R</i>	<i>T</i>	<i>R</i>	<i>T</i>	<i>R</i>	<i>T</i>
10	33 ± 3.8c	61 ± 8.1b	25 ± 16.5a	47 ± 6.7b	55 ± 1.9c	55 ± 1.9b	60 ± 12.4d	60 ± 12.4d
15	11 ± 4.3ab	51 ± 4.9b	22 ± 6.8a	52 ± 5.5b	35 ± 7.9b	43 ± 6.7b	39 ± 8.6c	39 ± 8.6c
20	6 ± 3.8ab	51 ± 3.8b	13 ± 5.9a	46 ± 8.2b	33 ± 13.2b	44 ± 8.5b	51 ± 4.9cd	51 ± 4.9cd
25	4 ± 3.3ab	53 ± 9.3b	19 ± 6.9a	54 ± 6.8b	60 ± 7.8c	68 ± 5.5c	53 ± 11.2cd	53 ± 11.2cd
30	0 ± 0.0a	58 ± 0.0b	11 ± 8.5a	50 ± 16.2b	33 ± 14.2b	52 ± 10.6b	18 ± 5.0b	18 ± 5.0b
35	23 ± 20.6bc	57 ± 18.2b	18 ± 9.0a	43 ± 3.8b	9 ± 6.3a	20 ± 5.5a	5 ± 1.9ab	5 ± 1.9ab
40	6 ± 2.6ab	10 ± 7.5a	19 ± 16.6a	20 ± 16.0a	13 ± 9.8a	13 ± 9.8a	0 ± 0.0a	0 ± 0.0a

Values (mean ± SE,  $n = 4$ ) within a column followed by the same letter are not significantly different at the  $P = 0.05$  level for the given salinity (Student–Newman–Keuls's test).

species, Sen and Chatterji (1968) found an absolute light requirement for germination. On the other hand, Thanos and Doussi (1995) indicated that seed germination of two Crete sage species is favoured by darkness. They showed that only germination of *S. pomifera* was slightly enhanced by light; however, germination of *S. fruticosa* was inhibited by white or red lights. Several reports concerning the light-temperature cofactor on the germination of *Salvia* species have been done. Comparing two *salvia* species, Oberczian and Bernath (1988) indicated that germination of *S. officinalis* was markedly influenced by light. These authors concluded that germination was inhibited for temperature below 20 °C and was stimulated above this temperature. However, germination of *S. sclarea* was indifferent to light (Oberczian and Bernath, 1988). For *S. hispanica*, germination responds to temperature and light differently and seeds are physiologically heterogeneous, containing positively photoblastic subpopulations at 15 °C and negatively photoblastic subpopulations at 35 °C; however, between 20 and 31 °C germination is light indifferent (Labouriau and Agudo, 1987).

Germination failure in desert regions is often a result of high salt concentration due to intense evapotranspiration. Our data show that seeds of *S. aegyptiaca* responded in two characteristic ways to salinity. First, germination was reduced, indicating that germination is inhibited by salt. Only at 300 mM NaCl, germination was completely limited. Second, seeds showed the phenomenon of “salt stimulation” after transferring to fresh water. *S. aegyptiaca* seeds have the ability to tolerate moderate salinity and if substrate salinity becomes reduced, under a suitable temperature regime, faster and higher germination was recorded (Table 1). This can be attributed to both ionic and osmotic effects (Song et al., 2005; Gorai and Neffati, 2007; Tlig et al., 2008). Under controlled conditions, the highest germination percentage was obtained in distilled water and variation in temperature substantially affected germination in both the saline and non-saline treatments. High temperatures and NaCl concentrations affected significantly germination patterns. Although higher salinity generally decreases germination, the detrimental effect of salinity is less severe at the optimum germination temperature (Al-Khateeb, 2006; Gorai and Neffati, 2007; Tlig et al., 2008). Salt stress decreased both the rate and percentage of germination of *S. aegyptiaca*. This result corroborates several other studies, revealing that halophytes, as glycophytes, are sensitive to salt

during the germination stage (Ungar, 1995; Katembe et al., 1998; Khan et al., 2002; Gorai and Neffati, 2007).

The seeds and/or fruits of many species of Asteraceae, Lamiaceae, Brassicaceae, Plantaginaceae and other families that frequently occur in desert habitats have an external mucilage layer (Gutterman, 2002; Huang et al., 2004; Zohary, 1962). This mucilage can provide some ecological benefits in these extreme conditions (Huang et al., 2008). This is the case for *S. aegyptiaca* where the outer surface of nutlets contains a pectinaceous mucilage layer that can imbibe a large amount of water when wetted. Yang et al. (2010) showed that intact achenes of *Artemisia sphaerocephala* exhibited higher germination percentages than those demucilaged as osmotic stress decreases either by drought or salinity. These authors concluded that mucilage presumably plays an ecologically important role in the life cycle of *A. sphaerocephala* by insuring under harsh conditions. In the present study, germination of *S. aegyptiaca* seeds was completely inhibited at 300 mM NaCl. However, their transfer to distilled water enhanced germination with relatively high percentage indicating that seeds keep the ability to germinate. It is possible that mucilage can serve as a kind of “filter” and/or “sorbent” preventing seed germination from harmful effect of salt (Yang et al., 2010).

From the present study, it can be concluded that *S. aegyptiaca* seeds germinate under a wide range of temperatures have the ability to tolerate salt stress and recover after exposure to NaCl solutions. Future studies would be focused on the role of the mucilage to better understand the ecophysiological strategies of plants to survive under natural environmental conditions.

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