

EFFECT OF DESICCATION AND SALINITY STRESS ON SEED GERMINATION AND INITIAL PLANT GROWTH OF *Cucumis melo*¹

Efeito da Dessecação e do Estresse Salino na Germinação de Sementes e Crescimento Inicial da Planta Cucumis melo

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ABSTRACT - Smellmelon, an annual invasive weed of soybean production fields in the north of Iran, reproduces and spreads predominately through seed production. This makes seed bank survival and successful germination essential steps in the invasive process. To evaluate the potential of Smellmelon to invade water-stressed environments, laboratory studies were conducted to investigate the effect of desiccation and salinity at different temperatures on seed germination and seedling growth of *Cucumis melo*. Seeds were incubated at 25, 30, 35 and 40 °C in the darkness in a solution (0, -0.2, -0.4, -0.6, -0.8, 1 and 1.2 MPa) of a salt (NaCl), and in a solution (0, -2, -4, -6, -8, -10, -12 bar) of PEG-6000 (Polyethylene glycol), in two separate experiments. The results showed that the highest percentage and rate of germination occurred at 35 °C in salt concentrations of 0, -0.2, -0.4 MPa and PEG concentrations of 0, -2, -4 bar. Increasing the concentration of salt (NaCl) and PEG limited germination, seedling growth and water uptake but increased the sodium content in the seedlings. No significant difference was observed among 0, -0.2 and -0.4 MPa of NaCl and among 0, -2 and -4 bar of PEG concentration at 35 °C. The negative effects of PEG were more than those of NaCl on germination percentage and germination rate. Increased stress levels lead to reduction of root and shoot length, and SVL of seedlings. Na⁺ content of seedling decreased with limited seedling growth of *C. melo*.

Keywords: germination, Osmotic potential, Cucurbitaceae, Seedling vigor index (SVL).

RESUMO - **Smellmelon**, uma planta invasora anual de campos de produção de soja no norte do Irã, se reproduz e se espalha predominantemente através de produção de sementes. Isso torna a sobrevivência do banco de sementes e a germinação bem-sucedida passos essenciais no processo invasivo. Para avaliar o potencial da planta **Smellmelon** de invadir ambientes com escassez de água, estudos de laboratório foram realizados para investigar o efeito da dessecação e da salinidade em diferentes temperaturas na germinação de sementes e crescimento de plântulas de **Cucumis melo**. As sementes foram incubadas a 25, 30, 35 e 40 °C na escuridão numa solução (0, -0,2, -0,4, -0,6, -0,8, 1 e 1,2 MPa) de sal (NaCl), e numa solução (0, -2, -4, -6, -8, -10, -12 bar) de PEG-6000 (polietilenoglicol), em dois experimentos separados. Os resultados mostraram que a percentagem e a taxa de germinação mais altas ocorreram a 35 °C nas concentrações de sal de 0, -0,2, -0,4 MPa e nas concentrações de PEG de 0, -2, -4 bar. O aumento da concentração de sal (NaCl) e PEG restringiu a germinação, o crescimento das plântulas e a absorção de água, mas aumentou o conteúdo de sódio nas plântulas. Nenhuma diferença significativa foi observada entre as concentrações de 0, -0,2 e -0,4 MPa de NaCl e 0, -2 e -4 bar de PEG a 35 °C. Com a diminuição da percentagem e da taxa de germinação, os efeitos negativos do PEG foram maiores do que aqueles do NaCl. O aumento dos níveis de estresse leva à redução do comprimento da raiz e parte aérea, e do IVP das plântulas. O teor de Na⁺ das plântulas diminuiu com o crescimento limitado das plântulas de **C. melo**.

Palavras-chave: germinação; potencial osmótico; Cucurbitaceae; Índice de vigor de plântulas (IVP).

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INTRODUCTION

Cucumis melo is a weedy summer annual form of the Cucurbitaceae family, with trailing prostrate stems to 5 m long or more and often forming large mats. It produces ellipsoid, golden yellow fruits which have a distinct odor (Anonymous, 2012). This weed is one of the weeds of soybean, cotton and corn production fields in the United States (Grichar, 2007). Tingle et al. (2003) reported that when smellmelon was allowed to compete with cotton for at least 6 weeks, yield was reduced by 7% compared to the weed-free check, but when smellmelon was allowed to compete for 10 to 12 weeks, cotton yield was reduced by 22 and 27%, respectively.

Salinity and drought are major environmental factors limiting plant growth and productivity. In Asia alone, 21.5 million ha are affected, 12 million ha of which are saline and 9.5 million ha are alkaline/sodic (Laütte et al., 2006). Salinity and drought stress biology and plant or seed responses at different levels have been discussed over two decades (Purcell & Specht, 2004; Bhagirath & Johnson, 2009). Germination and seedling growth were negatively affected by drought and salinity stresses (Purcell & Specht, 2004; Ebrahimi & Eslami, 2011). One of the major factors determining seed germination is soil moisture. The effects of salts on the functions of cell membranes and cell walls may affect the water potential of cytosol and cellular extensibility; thus, seed germination and seedling growth may be affected as well (Tobe et al., 2004). Previous research has shown that *C. melo* var. *dudaim* germination was reduced from 81 to 61% when the osmotic potential was reduced to -0.2 MPa, and it was further reduced to 48 and 7% with solutions of -0.4 and -0.6 MPa, respectively (Tingle & Chandler, 2003). Horak & Sweat (1994) showed that buffalo gourd (*Cucurbita foetidissima*) germination ranged from 77% (at 2 0.1 to 2 0.4 MPa) to 53% (at 2 0.8 MPa).

Most invasive plants primarily rely on seed dispersal and seedling recruitment for population establishment and persistence. Rapid spread of many invasive plants is frequently correlated with germination and dormancy patterns. Environmental factors,

such as temperature and soil solution osmotic potential, affect weed seed germination and emergence (Norsworthy & Oliveira, 2006). To understand why *C. melo* is so troublesome, it is important to gain a better understanding of the mechanism of how seeds germinate in response to different environmental factors, such as temperature and solution osmotic potential. A better understanding of *C. melo* seed germination and seedling growth improves the management of this weed by facilitating models that explore the influence of factors such as tillage and burial on germination and emergence.

C. melo grows in soybean fields in the north of Iran (Golestan province) and causes crop yield loss. Although it is a troublesome weed of soybean farming systems in the province of Golestan, no information is available about the effect of environmental factors on its germination. Therefore, the objectives of the study reported in this paper were (i) to evaluate the effect of salinity on seed germination, seedling growth, Na⁺ content and water uptake of seedlings and (ii) to determine the influence of PEG concentrations at different temperatures on seed germination, seedling growth and water uptake of *C. melo*.

MATERIAL AND METHODS

Matured aerial fruits from different plants of *Cucumis melo* were collected in August 2011 from several soybean fields in the city of Gorgan, in the North of Iran. Each fruit had about 300 seeds, and the size of each seed was about 5 mm.

Salt stress

To investigate the effect of salt stress and temperature on seed germination of *C. melo*, a factorial experiment was conducted in a completely randomized design with four replications. The factors were 6 concentrations of salt including 0, 0.2, 0.4, 0.6, 0.8, 1 and 1.25 MPa which were created using NaCl, and temperatures of 20, 25, 30, 35 and 40 °C. Each replication included 25 seeds that were incubated in moist paper towels with sodium chloride solutions at constant temperatures in the dark.

Desiccation stress

In order to evaluate the effect of desiccation on seed germination of *C. melo*, an experiment was carried out in a completely randomized design with a two-way factorial arrangement. The factors included desiccation levels (0, -2, -4, -6, -8, -10, -12 bar of PEG-6000) and temperatures of 25, 30, 35 and 40 °C based on the results reported by Michel & Kaufman (1973). Each replication included 25 seeds that were incubated in a moist paper towels with the solutions in the dark; the experiment was conducted with four replications.

In both experiments, seeds were observed daily and considered as germinated when the radicle was approximately 2 mm long or more. Germination rate (S) was calculated according to Equation 1 (Maguire, 1962).

$$S = \frac{E1}{N1} + \frac{E2}{N2} + \dots + \frac{En}{Nn} \quad (\text{eq. 1})$$

where E_n is the number of germinated seeds observed in the n^{th} daily count and N_n is the number of days after the seeds were put to germinate in the n^{th} count. Rootlet length, shootlet length and seedling vigor index (SVI) were calculated using Equation 2 at the end of the 10th day. In this equation, RL and SL stand for root length and shoot length, respectively, and n is the total germination on the 10th day.

$$SVI = \frac{(RL + SL)}{n} \quad (\text{eq. 2})$$

Evaluation of water uptake by seedlings

Seeds were incubated in both (NaCl and PEG) solutions after 10 days, seedlings were detached from the pericarp and were blotted dry with tissue paper, and the fresh weight of the seedlings from each towel was measured. After the seedlings were dried at 80 °C for 48 hours, their dry weight was determined. The fresh and dry weights of the seedlings were used to calculate water content per seedling.

Measurement of cation (Na⁺) content in seedlings

Seeds were incubated in a salt (NaCl) solution; after 10 days of incubation, seedlings

were detached from the pericarp, blotted dry with tissue paper and the fresh weight of the seedlings from each towel was measured. Next, the seedlings were dipped in water and quickly blotted dry again to remove any surface salts, and then dried at 80 °C for 48 hours. After the dry weight of the seedlings was determined, they were put in electric furnaces at 400 °C for 4 hours. Then, they were ground, digested with 5 mL of HCl 0.2 M on a hot plate, and finally dissolved in 50 mL of distilled water; the aqueous solution was analyzed for cation (Na⁺) content by a flame photometer (PFP7 GEMWAY). After that, calculations were made of cation content per seedling and cation concentrations in seedling water.

Statistical analyses

All experiments were carried out twice as a completely randomized design with four replications per treatment. The data of the experiments were pooled for analysis, as there was no time-by-treatment interaction. A functional three-parameter logistic model (Equation 3) (Chauhan et al., 2006a) was fitted to the germination values (%) obtained at different concentrations of NaCl or osmotic potential using SigmaPlot (version 11.0, SyStat Software, Inc., Point Richmond, CA, USA).

$$G(\%) = G_{\text{max}} / [1 + (X/X_{50})^{\text{Grate}}] \quad (\text{eq. 3})$$

In this equation, G represents total germination (%) at NaCl concentration or osmotic potential x , G_{max} is the maximum germination (%), x_{50} is the NaCl concentration or osmotic potential for 50% inhibition of the maximum germination, and Grate indicates the slope of the curve at x_{50} .

One-way ANOVA and Duncan's test were used to compare the multiple means. All statistical tests were conducted at $P < 0.05$ and the Statistical Analysis System (SAS) was used for analyzing data (SAS, 1989).

RESULT AND DISCUSSIONS

Effect of salt and PEG concentrations on germination

Seed germination time delayed with increased PEG and NaCl concentrations.



Therefore, germination started at different times in various PEG and NaCl concentrations and different temperatures (Figures 2 and 4). The three-parameter logistic model provided a satisfactory fit for the response of seed germination to NaCl and PEG concentrations (Figures 1 and 3). However, the increase in water stress with PEG and NaCl reduced the seed germination percentage, but it appears that drought has more effect than salt on germination indices. Seed germination percentage was significantly low at the highest NaCl concentration (1MPa) even at -8 bar of PEG (Figures 1 and 3). Germination percentage and rate were higher at 30 °C and 35 °C than at 25 °C and 40 °C for both NaCl and PEG concentrations (Figures 1, 2, 3 and 4). Therefore, the decrease in water potential gradient among seeds and their surrounding media by the effects of PEG 6000 and NaCl adversely affects seed germination. The reduction in seed germination percentage was higher for PEG compared to NaCl, that is, at the equivalents of osmotic potential, seed germination is better in NaCl than in PEG. These data suggest that smellmelon germination could occur over a wide range of soil moisture contents. Similar results were reported for *Mimosa pudica* (Souza Filho et al., 2001). Water stress causes a greater reduction in germination speed and accumulated germination of *Melaleuca quinquenervia* seeds than saline stress (Martins et al., 2011). Salinity stress up to 90 mM had no effect over seed germination of *Melilotus officinalis*, but its

germination decreased by increasing salt concentration (Ghaderi-Far et al., 2010). Seed germination of invasive weed Cadillo (*Urena lobata*) was reduced by water stress below

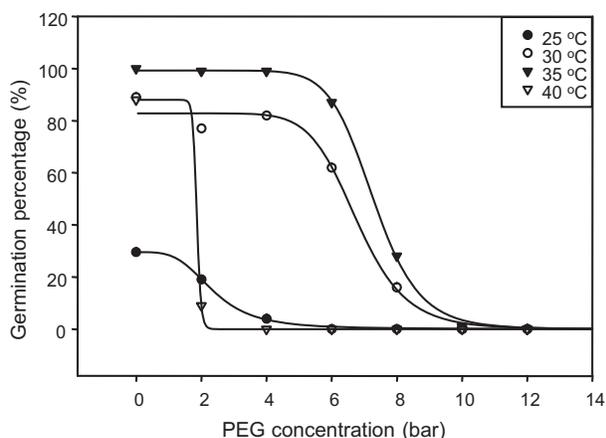


Figure 1 - Effect of water potential (PEG) and temperature on germination percentage of *C. melo* seeds.

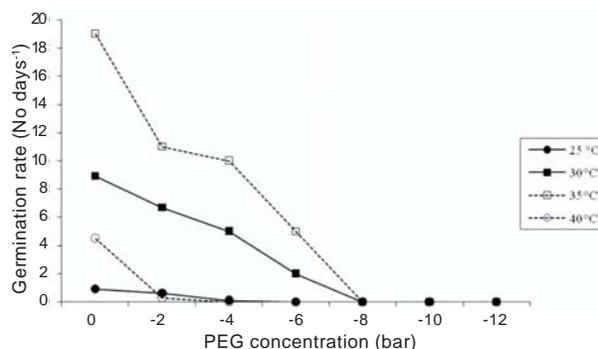


Figure 2 - Effect of water potential (PEG) and temperature on germination rate of *C. melo* seeds.

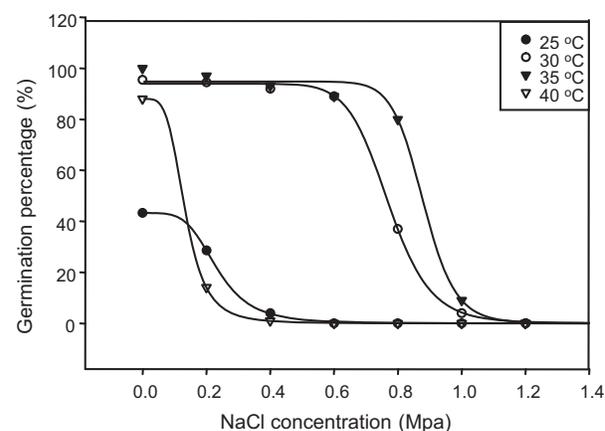


Figure 3 - Effect of NaCl concentrations and temperature on germination percentage of *C. melo* seeds.

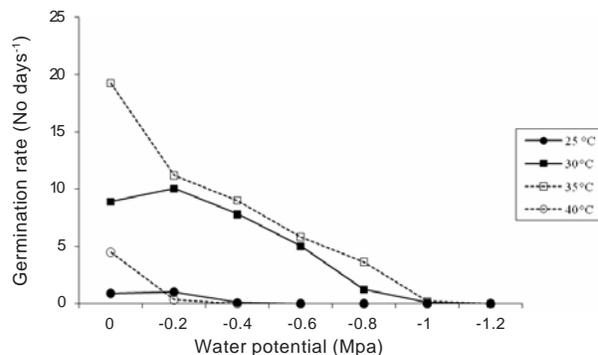


Figure 4 - Effect of NaCl concentrations and temperature on germination rate of *C. melo* seeds.

-0.2 MPa (Wang et al., 2009). Decreasing osmotic potential inhibited seed germination of both *Thymus daenensis* and *T. kotschyanus* (Khoshshokhan et al., 2012). Some other weed species, such as *Solanum sarrachoides*, were tolerant to low osmotic potential (Zhou et al., 2005). Smellmelon germination was reduced to 48 and 7% with solutions of -0.4 and -0.6 MPa, respectively (Tingle & Chandler, 2003). The parameter x_{50} of the fitted logistic model, which represents the NaCl concentration required for 50% inhibition of the maximum germination, was 0.8 Mpa. This is an additional indication of the high salt tolerance of this species during germination. The osmotic potential required for 50% inhibition of maximum seed germination (x_{50}) of *C. melo* (estimated by the fitted model) was considerably greater (-7 bar) than values reported for other weed species (Chauhan et al., 2006a, b, c; Eslami, 2011).

This parameter was only 89.6 for *S. oleraceus* (Chauhan et al., 2006a). Zia & Khan (2004) also reported that *Limonium stocksii*, a known halophytic species, had about 10% germination at 400 mM NaCl concentration.

Effect of salt and PEG on seedling growth

Increased stress levels lead to the reduction of root and shoot length. The low osmotic potential of 0.4 MPa of salt and -2 bar

of PEG increased root length at 35 °C compared to the control solutions. The subsequent low osmotic potential of 0.8 MPa of NaCl and -6 bar of PEG at 35 °C decreased root length. This reduction in root length was higher in the PEG solution than in the NaCl solution. The shoot length of *C. melo* differed under the different osmotic potentials of PEG and NaCl levels, and shoot length differed with temperature depending on stress conditions and stress levels (Tables 1 and 2). The results indicated that the seedling vigor index (SVL) decreased with increasing water potential. Moreover, this result showed that SVL was more reduced with the stress conditions that were obtained in the PEG concentration compared to the NaCl concentration. At 30 °C and 35 °C, SVL was higher than at 25 °C and 40 °C. Significant differences were detected at different temperatures and osmotic potentials (Table 3). Roots play an important role in plant survival during periods of drought, and drought resistance is characterized by extensive root growth and small reduction of shoot growth in drought stressed conditions (Guoxiong et al., 2002). Our result were supported by Zhou et al., (2005), who observed that the seedling length of *Solanum sarrachoides* significantly decreased as a result of increased salt concentration of NaCl. Root and shoot length of perennial ryegrass seedlings were also affected by different salinity application (Nizam, 2011).

Table 1 - Estimated parameters of the fitting model on the percentage germination of PEG concentrations at different temperatures

Temperature	Parameter				R ²
	G _{max}	X ₅₀	G _{rate}	P _{value}	
25° C	29.74(±1.2)	-2.3(±0.01)	3.75(±0.5)	0.0001	0.95
30° C	82.85(±1.8)	-6.7(±0.01)	9.04(±1.09)	0.0001	0.97
35° C	99.23(±1.5)	-7.2(±0.009)	10.43(±1)	0.0001	0.98
40° C	88(±2.4)	-1.5(±0.4)	8.6(±5.6)	0.0001	0.97

Table 2 - Estimated parameters of fitting model on the percentage germination of NaCl concentration at different temperatures

Temperature	Parameter				R ²
	G _{max}	X ₅₀	G _{rate}	P _{value}	
25° C	43.23(±1.2)	0.23(±0.007)	4.35(±0.5)	0.0001	0.98
30° C	93.95(±2.02)	0.77(±0.012)	11.71(±2.45)	0.0001	0.97
35° C	94.8(±1.7)	0.88(±0.01)	17.5(±2.3)	0.0001	0.97
40° C	88(±2.58)	0.13(±0.04)	4.12(±3.6)	0.0001	0.97



Table 3 - Effects of water potentials on germination traits, vigor index and water uptake of *C. melo* at different temperatures

T	25 °C				30 °C				35 °C				40 °C				
	SL	RL	SLV	WU	SL	RL	SLV	WU	SL	RL	SLV	WU	SL	RL	SLV	WU	
Drought	0 bar	7.41c	2.62fg	1.38	0.16gh	15.66a	5.33ef	0.94	1.76a	11.05b	6.85cde	0.71	1.34b	1.008gh	0.7g	0.06hi	
	-2 bar	4.9cde	6.06de	2.3	0.22g	6.25cde	9.22bc	0.79	1.13c	6.8cd	12.42a	0.77	1.23c	0h	0g	0.06i	
	-4bar	1.26fgh	1.608g	2.86	0.02i	4.19def	8.71bcd	0.63	0.84d	4.5cde	9.85bc	0.57	0.91d	0h	0g	0i	
	-6bar	0h	0g	0	0i	1.4fgh	6.05de	0.48	0.34f	3.2efg	9.98ab	0.62	0.47e	0h	0g	0i	
	-8bar	0h	0g	0	0i	0.25gh	2.53fg	0.69	0.022i	0.2gh	4.85ef	0.73	0.44i	0h	0g	0i	
	-10bar	0h	0g	0	0i	0h	0	0	0i	0h	0	0	0	0h	0g	0i	
	0MPa	9.91bc	3.62bcde	5.5 a	0.18e	14.83 a	5.33abcd	0.97c	1.76a	10.7 b	6.85 ab	0.7 cd	1.35 b	0.8 e	0.9 e	0.044 cde	0.06 e
	-0.2 MPa	6.5 d	5.12abcd	1.63 b	0.61 d	7.7bcd	5.7 abc	0.55 cde	1.85 a	9.5 bc	6.9 ab	0.65 cd	1.89 a	0 e	0 e	0 e	0 e
	-0.4 MPa	1.12 e	1 e	1.011 c	1.48 b	6.35 d	5.3abcd	0.5 cde	1.02 c	7.6bcd	7.5 a	0.59 cde	1.73 a	0 e	0 e	0 e	0 e
	-0.6 MPa	0 e	0 e	0 e	0 e	2.3 e	1.87 de	0.2 de	1.04 c	3.6 e	3.5 cde	0.28 de	1.04 c	0 e	0 e	0 e	0 e
-0.8 MPa	0 e	0 e	0 e	0 e	2.24 e	2 de	0.53 cde	0.15 e	2.25 e	2.26 de	0.2 cd	0.51 d	0 e	0 e	0 e	0 e	
-0.1 MPa	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	0 e	
Salinity																	

Effect of salt and PEG on seedling water uptake

Seedling water content was significantly lower in the PEG treatment than in the salt treatment. Among salt treatments (-0.2 and -0.4 MPa), no significant differences were detected in water contents. The highest water contents in both NaCl and PEG treatments were detected in -0.2 & -0.4 MPa and -2 & -4 bar, respectively. In both NaCl and PEG treatments, water content decreased with increasing water potential. Moreover, greater reduction in water content was obtained in PEG concentrations compared to NaCl concentrations. Significant differences were detected at different temperatures and osmotic potentials (Table 1). Dodd & Donovan (1999) reported that high NaCl contents and water deficient condition reduce germination because of limited water uptake by the seeds. The greater tolerance of salinity during the germination phase might, in part, be the result of a lower sensitivity to high tissue Na⁺ concentrations (Khajeh Hosseini et al., 2002). Reduction in the seed water content caused by low media water potential decreased the activity of hydrolytic enzymes such as α -amylase, proteases and lipases, which are responsible for hydrolyzing cotyledons reserves required for providing energy in the early stages of seed growth by respiration (Aroca et al., 2012). Kaydan & Yagmur (2008) reported that high NaCl concentrations caused lower water uptake by seeds and, consequently, germination decreased. The result of their research is similar to the results of this study. In the same way, Munns (2002) reported that water stress reduces the ability of plants to absorb water.

Effect of salt on cation (Na⁺) content in seedlings

A marked increase in Na⁺ concentration in seedling water content was detected when seedlings were treated with salt NaCl. Higher cation content was observed at 35 °C and 30 °C than at 25 °C and 40 °C. Sodium content per seedling was significantly higher in -0.6 MPa of NaCl concentration at 35 °C (Figure 5). Salinity might negatively affect some important physiological processes in

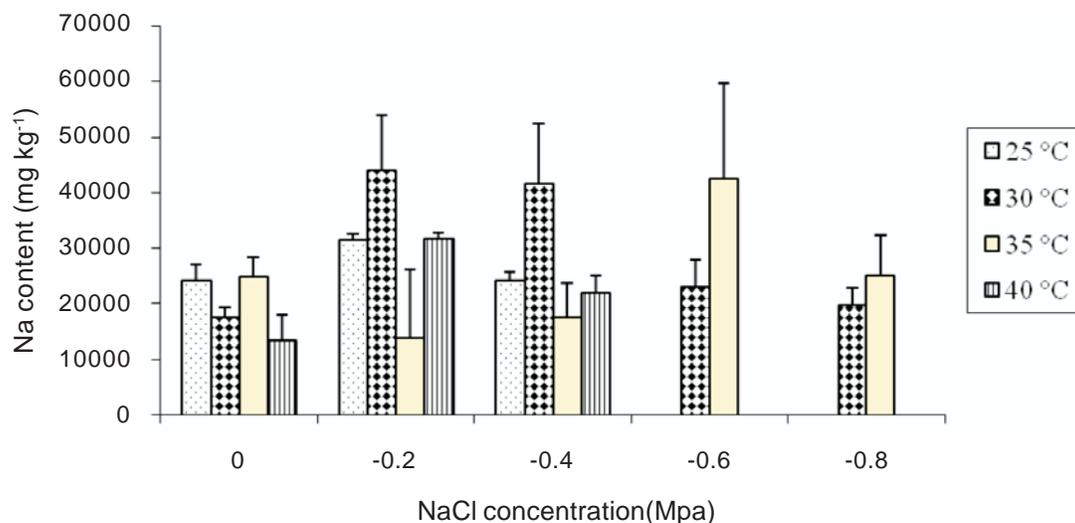


Figure 5 - Effect of NaCl concentrations and temperature on Na content of *C. melo* seedlings.

plants. Additionally, sodium ions can alter soil structure and fertility by replacing calcium and magnesium in anion exchange and this leads to nutrient stress and water stress (Rao et al., 2008). Addition of 5 mMolal CaCl₂ to NaCl caused a significant increase in Na⁺ content per seedling of *Haloxylon ammodendron* (Tobe et al., 2004). NaCl affects the permeability of the plasma membrane and increases the influx of external ions and the efflux of cytosolic solutes (Cuin et al., 2011) in plant cells. Concentrations of shoot Na⁺, root Na⁺, and root K⁺ in *Sporobolus virginicus* (increased with increasing salinity (Marcum & Murdoch, 1992). Ramadan (2001) observed similar patterns of salt secretion in *S. spicatus*.

The results indicate that *C. melo* is able to germinate and emerge over a wide range of temperatures and salinity values, and desiccation partially explains why this plant is one of the most important weeds of soybean fields in the north of Iran. Soil temperatures usually reach 35 °C in May in the province of Golestan. This time coincides with that of soybean planting and explains why smellmelon germination and subsequent emergence can interfere with soybean production. According to the results of these two experiments, it can be concluded that smellmelon tolerates up to -8 MPa (about 10.168 g L⁻¹ ≈ 13 ds m⁻¹) salinity level at the germination and early seedling growth stages. Also, it is relatively tolerant to

drought stress at the germination and early seedling growth stages. This characteristic could not only increase geographic spread but also allow germination during adverse environmental conditions.

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