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GERMINATION AND SEEDLING GROWTH OF *ACACIA RADDIANA* AND *ACACIA NILOTICA* AT DIFFERENT LEVELS OF SALINITY AND DROUGHT UNDER *IN VITRO* CONDITIONS

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AZIZ, A. A. S., OMARI, M. A. & KAFAWIN, O. M. 2001. Germination and seedling growth of *Acacia raddiana* and *Acacia nilotica* at different levels of salinity and drought under *in vitro* conditions. An *in vitro* experiment was conducted to study seed germination and growth of *Acacia raddiana* and *A. nilotica* seedlings under induced water deficit and salinity conditions. Different concentrations of either sodium chloride or mannitol (0.1, 0.2, 0.3 and 0.4 M) were used to induce salt and drought stresses. *In vitro* seed germination of *A. raddiana* and *A. nilotica* decreased as the salinity and drought stress increased. *Acacia raddiana* and *A. nilotica* survived *in vitro* a concentration of 0.4 M NaCl; thus the two species could be classified as moderately salt tolerant. They also survived *in vitro* a high concentration of mannitol. Therefore, the two species can be classified as drought-enduring plants. *Acacia raddiana* was more tolerant to drought stress than *A. nilotica*.

Key words: *Acacia raddiana* - *Acacia nilotica* - *in vitro* - salinity - drought - seed germination - seedling growth

AZIZ, A. A. S., OMARI, M. A. & KAFAWIN, O. M. 2001. Percambahan dan pertumbuhan anak benih *Acacia raddiana* dan *Acacia nilotica* pada peringkat kemasinan dan kemarau yang berbeza di bawah keadaan *in vitro*. Satu uji kaji *in vitro* dijalankan untuk mengkaji percambahan biji benih dan pertumbuhan anak benih *Acacia raddiana* dan *A. nilotica* di bawah keadaan kekurangan air teraruh dan keadaan kemasinan teraruh. Kepekatan sodium klorida atau mannitol yang berbeza (0.1, 0.2, 0.3 dan 0.4 M) digunakan untuk mengaruh tegasan garam dan kemarau. Percambahan biji benih *in vitro* bagi *A. raddiana* dan *A. nilotica* berkurangan apabila kemasinan dan tegasan kemarau bertambah. *Acacia raddiana* dan *A. nilotica* memandirikan *in vitro* kepekatan sebanyak 0.4 M NaCl; oleh itu kedua-dua spesies dapat diklasifikasikan sebagai pokok yang toleran garam. Spesies ini juga memandirikan *in vitro* kepekatan mannitol yang tinggi. Oleh yang demikian, kedua-dua spesies dapat diklasifikasikan sebagai pokok yang tahan kemarau. *Acacia raddiana* adalah lebih toleran kepada tegasan kemarau berbanding dengan *A. nilotica*.

Introduction

In dry zones *Acacia* plays an important role as browse plants and as emergency fodder; the wood is useful for fuel, while the trees provide shade and shelter and are important in soil conservation (Doran *et al.* 1983). In Wadi Araba, Jordan, *Acacia raddiana* and *A. nilotica* grow naturally. However, few *A. raddiana* trees remain and the existence of *A. nilotica* has become rare.

In arid and semi-arid zones that receive less than 300 mm and up to 600 mm rainfall respectively, water and salts are the main environmental factors influencing plant growth (Goor & Barney 1976). According to Ashkenazi (1995) and Bensada *et al.* (1996), changes in water availability and water quality seem to be the main reason for *Acacia* mortality in Wadi Araba. Regeneration of these trees in nature is quite low (Dewan *et al.* 1992).

Plant reaction to moisture stress embodies both physical and physiological components (Leopold & Kriedemann 1975). In *A. raddiana* and *A. tortilis,* partial defoliation occurs during the summer. This defoliation covers 40–50% of the tree and in some cases the tree turns completely bare (Halevy & Orshan 1973). The leaf water potential of sorghum decreases, plant stomata close abruptly but not completely, and leaf diffusion resistance increases with decreasing soil water potential (Blume & Sullivan 1974).

According to Bradburg (1990), the mean relative growth rate decreases in non-stressed and water stressed plants of *A. nilotica* and *Sesbania sesban* as a result of a decline in mean leaf area, but the decrease in water stressed plants is greater. Relative growth rate was depressed by both stress factors (polyethylene glycol 6000 or NaCl), more severely for maize than sorghum (Erdei & Taleisnik 1993). Water stress significantly decreases the rate of leaf production, live and total leaf number, total length of the central leaflet on live leaves per plant, branch and root segment production and top and root dry weights (Fedorenko *et al.* 1995).The decrease in rate and percentage of germination as a result of moistening the seeds with either NaCl or mannitol can be ascribed to the osmotic effect of the solution on the seeds (Ayers 1952).

With regard to the salt tolerance of plants, two different theories have been advanced: the harmful effect of salts on plants is due to the osmotic pressure of the external solution according to one, and according to the other, the toxic action of the salts (FAO 1973).

The objective of this work was to study the effects of drought and saline conditions on seed germination and survival of *A. raddiana* and *A. nilotica* plantlets under *in vitro* conditions.

Materials and methods

This research was conducted at the Plant Tissue Culture Laboratory of the University of Jordan. The *A. raddiana* and *A. nilotica* seeds were obtained from the Department of Forestry and Rangeland, Ministry of Agriculture, Jordan. The seeds were collected in 1995. To improve the germination percentage, they were treated by soaking for 15 min in 96% sulphuric acid and then rinsed three times in sterile distilled water with shaking on a rotary shaker under a laminar air flow cabinet to remove the acid completely.

Experimental I

In vitro seed germination of *Acacia raddiana* and *A. nilotica*

The experiment was designed to study *in vitro* seed germination of *A. raddiana* and *A. nilotica* under induced water deficit and salinity conditions. Murashige & Skoge medium (MS) (Murashige & Skoge 1962) was used and supplemented with the following osmotic agents: NaCl (0.1, 0.2, 0.3 and 0.4 M) and mannitol (0.1, 0.2, 0.3 and 0.4 M). These osmotic agents were completely dissolved before attaining the final value. The control was prepared by using MS medium without osmotic agents. Bacto-agar (0.5%) was used to solidify the medium. The pH was adjusted to 5.7 with 1 M KOH or 1M HCl. The media was dispensed into 50 ml plastic pot and sterilised at 121°C for 20 min at 15 psi in an autoclave. Five disinfected seeds were placed in each pot in a growth chamber. The germination treatment was conducted in the growth chamber at 23 ± 2 °C in 16-h photoperiod under cool-white florescent tube (50–70 μM m^2 s^{-1}). Each treatment consisted of 10 pots. A sample of medium was taken from each treatment to determine the osmolality using a Wescur 5500 vapor pressure osmometer. After two months of placing the seeds in pots the percentage of germination was recorded. Seeds were considered germinated when the radical emerged (Bell *et al.* 1995).

Experiment II

Seedling growth of *Acacia raddiana* and *A. nilotica*

In this experiment the effects of water deficit and salinity conditions on growth were evaluated in the two species of *Acacia*. Seeds of *A. raddiana* and *A. nilotica* were treated as described earlier to prevent the inhibitory effect of seed coats on germination. Seeds were germinated on MS basal medium supplemented with 3% sucrose, 0.1g/l myo-inositol and 0.5% bacto-agar.The pH of medium was adjusted to 5.7. After 30 days, plants of the two species were transferred to other media, supplemented with different concentrations of NaCl (0.0, 0.1, 0.2, 0.3 and 0.4 M) and mannitol (0.0, 0.1, 0.2, 0.3 and 0.4 M). Before the transfer, roots of plants were rinsed with autoclave-distilled water to remove the media and blotted with sterile tissue paper, and then the fresh weight was recorded using a sterilised balance. Then plants were cultured on sterile MS medium and the plant height was recorded. Before using the medium, it was dispensed into 10 ml in glass Pyrex test tube (25 x150 mm), plugged with polypropylene caps and sterilised at 121 °C for 20 min at 15 psi. Each treatment consisted of 12 test tubes and each test tube contained one plant. The test tubes were sealed with parafilm and placed under continuous shaking on a rotary shaker at 20 rpm in the growth chamber. This experiment was conducted in the growth chamber at 23 ± 2 °C under cool-white florescent tube (50–70 μM m^2 s^{-1}). After thirty days the following parameters were recorded: fresh weight, plant length, leaf number and relative growth rate [defined by $(\ln H_2 - \ln H_1)/(t_2 - t_1)$, where H_1 and H_2 refer to the height of the plants at the start (t_1) and finish (t_2) of the experiment (Radford 1967)].

Experimental design and statistical analysis

The split-plot arrangement in complete randomized design (CRD) was used for the first and second experiments. Analysis of variance (ANOVA) and the least significant difference (LSD) was calculated at probability level 0.05 using the Statistical Analysis System (SAS) (SAS Institute 1994).

Results and discussion

In vitro seed germination of Acacia raddiana and Acacia nilotica

Mannitol and NaCl were used to simulate the effect of drought and salt stress. Water stress and species had significant effects on total seed germination percentage as determined by ANOVA. The germination percentage of *Acacia raddiana* and *A. nilotica* decreased as the osmotic concentration increased except in the mannitol treatments with *A. raddiana* (Table 1). In *A. raddiana* the germination percentage (58.33%) decreased sharply at 0.2 M NaCl compared to the control, and it was 1.67% with 0.4 M NaCl. *Acacia nilotica* showed a decrease in germination percentage from 96.67% in the control down to 3.33% with 0.4 M NaCl and 16.67% with 0.4 M mannitol. There were no significant differences between the control and treatments with 0.1 M NaCl, 0.1 M mannitol and 0.2 M mannitol.

Table 1. Germination percentage of *Acacia raddiana* and *A. nilotica in vitro* at different levels of NaCl and mannitol after two months

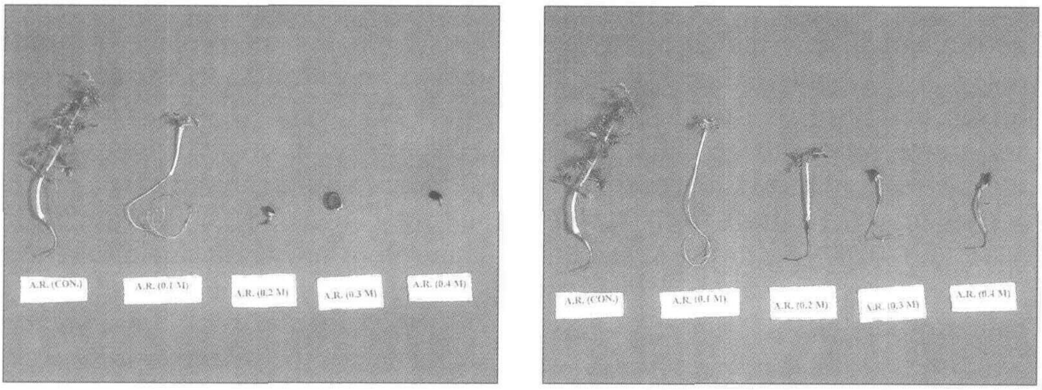
Treatment	Osmolality (moms kg^{-1})	Germination (%)	
		Acacia raddiana	*Acacia nilotica*
Control	212	83.33 b*	96.67 a
NaCl (M)			
0.1	372	81.67 b	93.33 a
0.2	515	58.33 d	50.00 d
0.3	613	31.67 e	10.00 fg
0.4	731	1.67 g	3.33 g
Mannitol (M)			
0.1	259	75.00 bc	96.67 a
0.2	342	71.00 c	95.00 a
0.3	402	80.00 bc	75.00 bc
0.4	548	78.00 bc	16.67 f

*: Means in columns followed by similar letter(s) are not significantly different at p = 0.05.

Germination was less affected by mannitol treatments than salt treatments, which can be attributed to injury caused by the accumulation of toxic amounts of chloride within the seed; this is in agreement with the observation of Ayers (1952). In all mannitol treatments, the germination percentage of *A. raddiana* ranged between 71 and 80% and there were no significant differences between them. Therefore, *A. raddiana* was more tolerant of drought stress than *A. nilotica*. Yaung *et al.* (1983), as cited by Qi and Redmann (1993), reported that low water potential delays initiation of seed germination, slows the rate of germination and decreases the final germination percentage.

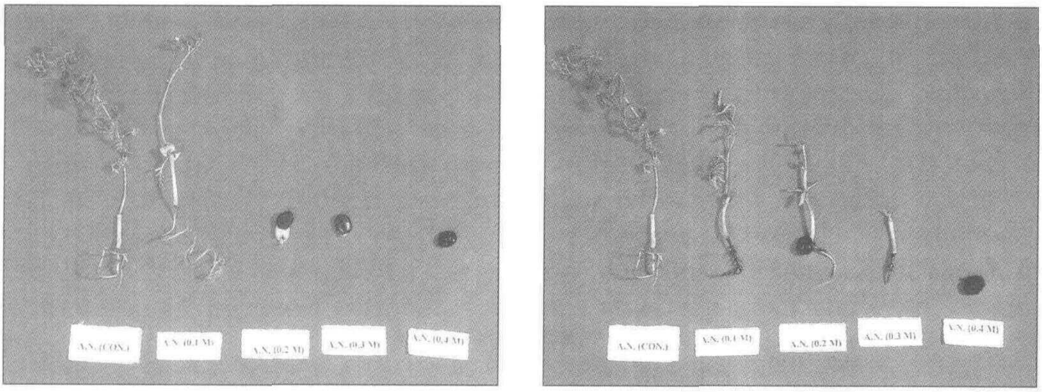
The control seeds of *A. raddiana* and *A. nilotica* gave the highest germination percentages of 83.33 and 96.67% respectively (Table 1), values that are higher than any previously reported for the same species (FAO 1973, Goor & Barney 1976).

Seedlings of both species continued to develop in the control and in 0.1 M NaCl and mannitol treatments. However, increasing concentrations of the osmoticum agents resulted in abnormal or stunted growth (Figures 1, 2).



(a) (b)

Figure 1. *Acacia raddiana* germination on MS media under different levels of (a) salinity stress, and (b) drought stress



(a) (b)

Figure 2. *Acacia nilotica* germination on MS media under different levels of (a) salinity stress, and (b) drought stress

Seedling growth of Acacia raddiana and A. nilotica

The growth responses of seedlings produced from seeds of *A. raddiana* and *A. nilotica* under different concentrations of NaCl and mannitol after 30 days are shown in Table 2 and Figures 3 and 4. In *A. raddiana,* fresh weight, number of leaves seedling^{-1} and relative growth rate (RGR) decreased with increasing salinity and drought stress. Compared to the control, the only increase was seen in the 0.1 M NaCl treatment (where only the fresh weight was significantly higher) and in the fresh weight of the 0.2 M NaCl and number of leaves seedling^{-1} of the 0.1 M NaCl and 0.2 M NaCl treatments (where there was no significant difference between all the parameters and the control). With increasing concentration of NaCl, despite the declining trend in all the parameters, the decreases were not similarly significant. For example, the fresh weight decreased significantly from 0.1 M to 0.3 M NaCl and number of leaves seedling^{-1} decreased significantly from 0.2 M to 0.4 M NaCl. Except for the number of leaves seedling^{-1} with 0.1 M mannitol, no significant differences were recorded for the fresh weight, number of leaves seedling^{-1} and RGR among the mannitol treatments on *A. raddiana* despite the decreasing trend with increasing concentration. The growth parameters of seedlings grown on saline media were greater than those of seedlings grown on mannitol.

Table 2. Growth response of seedlings produced from seeds of *A. raddiana* and *A. nilotica* under different levels of NaCl and mannitol on MS medium

Species	Treatment	Osmolality (moms kg^{-1})	Fresh weight (g)	Number of leaves seedling^{-1}	Relative growth rate (RGR) (cm day^{-1})	Relative growth rate (%)
A. raddiana	Control	219	0.38 hi *	5.42 ef	0.083 de	-
	NaCl (M)					
	0.1	388	0.55 g	5.92 cde	0.09 cd	108.4
	0.2	516	0.41 h	5.83 cde	0.075 ef	93.7
	0.3	709	0.34 i	3.67 g	0.056 fg	67.5
	0.4	874	0.15 i	2.17 h	0.029 ij	34.9
	Mannitol (M)					
	0.1	326	0.25 j	4.08 g	0.037 hi	44.5
	0.2	418	0.22jk	2.67 h	0.029 ij	34.9
	0.3	552	0.16 kl	2.08 h	0.020 j	24.0
	0.4	589	0.16 kl	2.08 h	0.019 j	22.9
A. nilotica	Control	219	1.15 a	9.17 a	0.14 a	-
	NaCl (M)					
	0.1	388	0.82 c	6.50 c	0.10 bc	71.4
	0.2	516	0.79 cde	6.00 de	0.11 b	78.6
	0.3	709	0.78 cde	5.67def	0.09 cd	64.2
	0.4	874	0.69 f	5.08 f	0.06 fg	42.8
	Mannitol (M)					
	0.1	326	0.92 b	8.42 b	0.08 de	57.0
	0.2	418	0.74 def	5.92 de	0.07 ef	50.0
	0.3	552	0.72 ef	6.17 cd	0.05 gh	35.7
	0.4	589	0.71 ef	5.58 ef	0.04 hi	28.5

*: Means in columns followed by the same letters are not significantly different at $p = 0.05$.

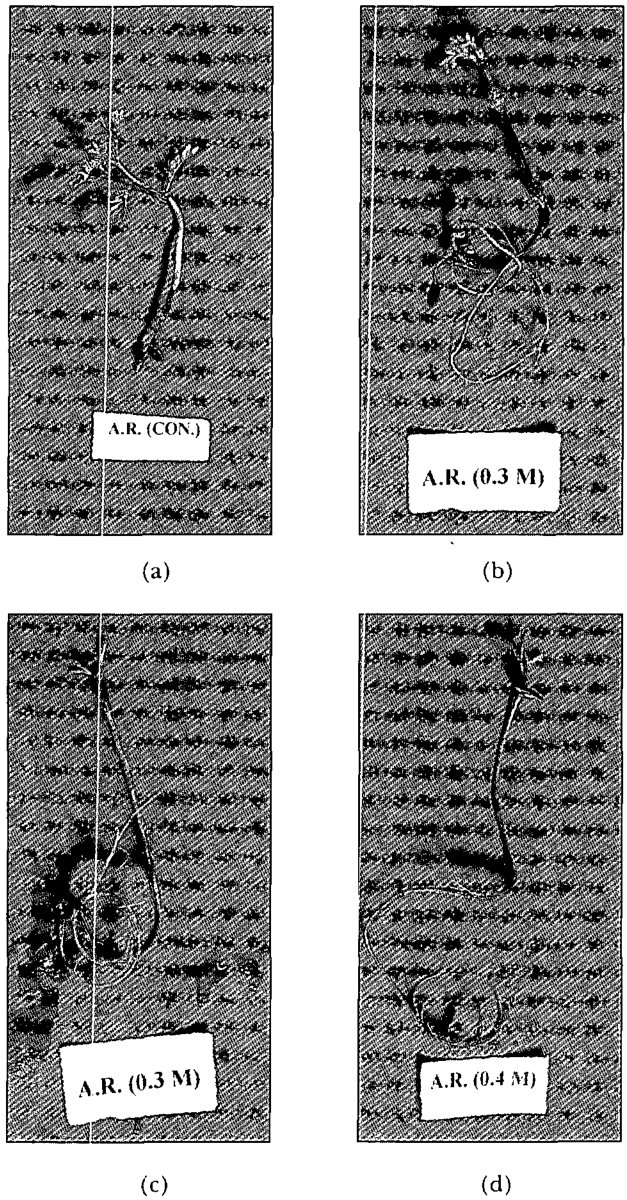


Figure 3. *Acacia raddiana* on MS media under (a) control, (b) 0.3 M NaCl, (c) 0.3 M mannitol, and (d) 0.4 M mannitol

Acacia nilotica showed a decrease in fresh weight, number of leaves seedling^{-1}, and relative growth rate as osmoticum increased. This decrease was significant for fresh weight at 0.1, 0.2 and 0.4 M NaCl and at 0.1 M and 0.2 M mannitol. From 0.2 M to 0.4 M mannitol, the decrease was not significant. For the number of leaves seedling^{1} the difference was significant at 0.1M and 0.2 M for NaCl and mannitol, but it was not significant from 0.2 M to 0.4 M NaCl and from 0.2 M to 0.3 M mannitol.

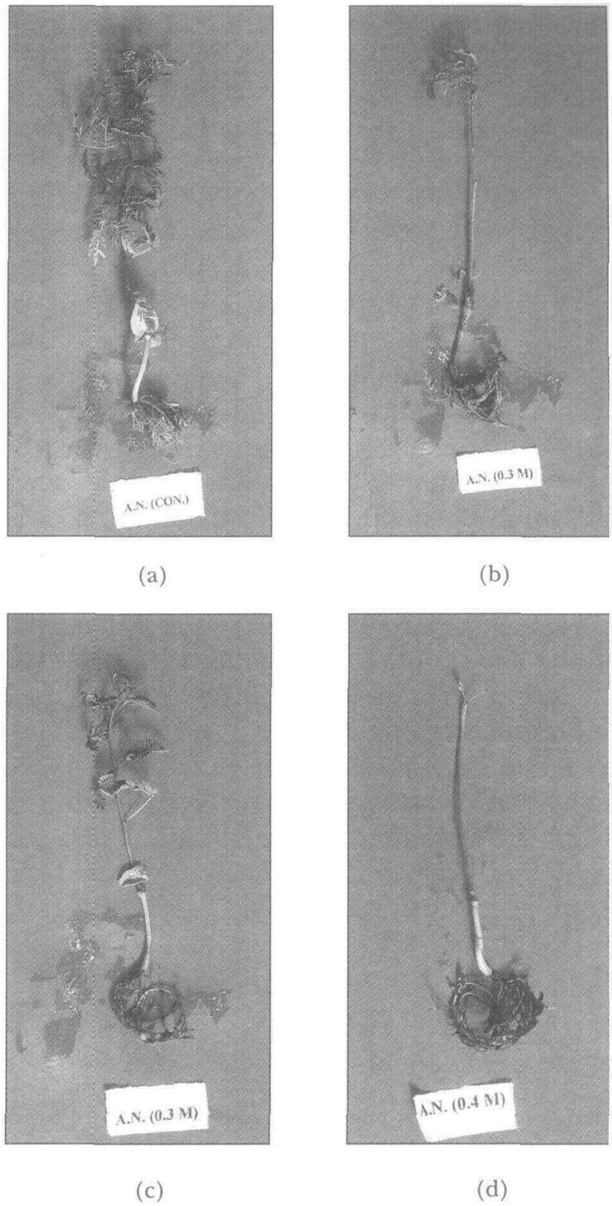


Figure 4. *Acacia nilotica* on MS media under (a) control, (b) 0.3 M NaCl, (c) 0.3 M mannitol, and (d) 0.4 M mannitol

The value of relative growth rate declined as the NaCl and mannitol concentrations increased, but this decrease was significant only for 0.1 M and 0.4 M NaCl and 0.1 M and 0.3 M mannitol. Relative growth rate values obtained for plants are also expressed as percentages of the control to reflect the inhibition of growth potential caused by the treatments. RGR was 93.7% of the control for *A. raddiana* and 78.6% for *A. nilotica* at 0.2 M NaCl. RGR decreased to 34.9 and 50.0% at 0.2 M mannitol for the two species respectively.

Under saline and drought stresses with up to 0.4 M concentration of either NaCl or mannitol, plants of both species survived. Although the growth parameters in both species declined as sodium chloride increased, survival was still observed at 0.4 M. Thus, these two species may be classified as moderately salt tolerant (Greenway & Munns 1980). *Acacia raddiana* showed an increase in fresh weight, number of leaves and RGR at 0.1 M NaCl (Table 3). This result is in agreement with the finding of Ashkenazi (1995) that the *Acacia* species require low soil salinity of less than 0.3%.

The most obvious morphological difference in salt grown plants, apart from decreased growth, was reduction in leaf number (especially for *A. nilotica*) (Table 3). This reduced leaf number could be the result of excess Na^+ and Cl^- ions inducing chlorosis and death of expanded leaves. Such injury is known in many woody plants (Bernstein 1975) and leads to decrease in photosynthetic leaf area. As a result, the production of carbohydrate declines and productivity falls below a level capable of sustaining further growth (Nabil & Courdret 1995).

In mannitol grown plants, the reduction in leaf number has advantage in reducing transpiration. Thus, *A. raddiana* and *A. nilotica* may be classified as drought-enduring plants. They do not conserve water, but lose their leaves, and although drying to low levels, still survive (Parker 1968). The relative growth rate of water-stressed plants of both species decreased as the osmoticum increased. Similar results were obtained by Bradburg (1990) and by Nabil and Courdret (1995).

This study shows that *A. raddiana* and *A. nilotica* are affected by increasing salinity and drought stress which cause a decrease in the seed germination percentage and growth parameters of seedlings. These two species can be classified as drought- and salt-tolerant although salt stress appears to negatively affect *A. raddiana* only at concentrations of NaCl greater than 0.2 M.

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