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EFFECT OF NACL SALINITY ON THE GERMINATION AND SEEDLING GROWTH OF SOME MEDICINAL PLANTS

ZAHIR MUHAMMAD AND FARRUKH HUSSAIN

Department of Botany,
University of Peshawar, Peshawar, Pakistan

Abstract

Seeds of *Lepidium sativum* L., *Linum usitatissimum* L., *Plantago ovata* Forssk., and *Trigonella foenum-graecum* L. were tested in 0.05 (Control), 2.5, 5.0, 7.5, 10.0, 12.5, or 15.0 dS/m concentration of NaCl. ANOVA revealed highly significant differences for plumule growth while germination percentage, radicle growth, seedling fresh and dry weight and moisture contents showed non-significant variation under various salt concentrations. However, the differences among the species for all the parameters studied were highly significant. The findings suggest that these medicinal species might tolerate moderate levels of salinity and can be tried for cultivation on marginal salted soils.

Introduction

Soil salinity is a world wide problem in arid and semi arid regions, which drastically alters both physical and biological environments (Eagleshman & Ayanaba, 1984). Many social and economic problems are caused by salinity that affects the growth, productivity and distribution of plants. Delayed germination, high rate of seedling mortality, stunted growth and reduced yield are some of the most common effects of salted soils.

Out of 20.2 million hectares of cultivated land in Pakistan, 6.8 million hectares are affected with salinity (Anon., 2002). The increasing population of over 3% demands more food, shelter and resources. Of the many available approaches, biological approach appears to be long lasting and cheap solution that means to live with salinity. Introduction of salt tolerant plants on such soils will not only provide economic returns but also likely to improve these unproductive soils.

Research in relation to the effect of salinity has mostly been carried out on agricultural, forage and fuel wood species. However, little work has been done for exploring the possibility of using salted habitats for the cultivation of medicinal plants. The available literature includes the effects of salinity on the germination and seedling growth of *Linum usitatissimum* (El-Nakhlaway & El-Fawal, 1989), *Plantago* spp., (Mackova *et al.*, 1988), *Trigonella foenum-graecum* (Shadded & Zaidan, 1989; Ali *et al.*, 1992), *Helianthus annuus* (Khan *et al.*, 1994; Daniela *et al.*, 2004; Mutlu & Bozcuk, 2007), *Brassica* spp., (Hussain & Ilahi, 1992; Ibrar *et al.*, 2003; Ulfat *et al.*, 2007), *Sesamum* (Datta *et al.*, 1990), *Glycine max* (Essa, 2002), *Vigna* spp., (Nandawall *et al.*, 2000; Jabeen *et al.*, 2003) and some other medicinal plants (Ibrar & Hussain, 2003; Hanselin & Eggen, 2005). All these workers suggest that medicinal plants might be grown on saline soils. *Lepidium sativum*, *Linum usitatissimum*, *Plantago ovata* and *Trigonella foenum-graecum* are considered medicinally important plants.

Present study was conducted to see the possible effects of NaCl salinity on the germination and seedling growth of these medicinal plants. The findings might help enhancing the medicinal wealth of Pakistan by utilizing the otherwise non-productive saline habitats.

Materials and Methods

Seeds of *Lepidium sativum* Linn., *Linum usitatissimum* Linn., *Plantago ovata* Forssk. and *Trigonella foenum-graecum* Linn., were obtained from the open market. They were grown in 0.05 (Control), 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 dS/m saline solution of NaCl. Seeds were placed on twice folded Whatman # 1 filter paper seed beds in Petri dishes. Each Petri dish was provided with 5 ml of the respective salt concentration. There were 10 replicates, with 10 seeds in each replicate.

The glassware prior to use was thoroughly washed with tap water followed by a rinsing with distilled water and then sterilized at 170^{o}C for 4 hours. The dishes were incubated at 25^{o}C. Germination, length of plumule and radicle was determined after 96 hours. Forty seedlings from each of the treatments were randomly selected for fresh and dry weight determination. Seedlings were dried at 65^{o}C for 72 hours. The moisture contents of seedlings were determined on oven dry basis (Hussain, 1989). The results were subjected to ANOVA (Steel & Torie, 1980).

Results and Discussion

Effect on germination: ANOVA revealed that the effects of various concentration of NaCl salinity on the seed germination were insignificant. However, the differences among different test species were highly significant (Table 1). Table 2 shows that maximum average value for germination i.e., 82.75% obtained in control declined smoothly with increasing concentration to 59.50% at the highest concentration applied. Of the test conditions, the average germination was above 70% at 10.0 dS/m and at lower concentrations. The maximum average germination i.e., 83.43% was recorded in *Linum* that was followed by *Trigonella* (75.57 %), *Lepidium* (70.86%) and *Plantago* (58.57%) (Table 2).

Germination and seedling establishment are generally most sensitive stages in plant life (Ashraf *et al.*, 1986). Salinity causes osmotic stress (Nandawal *et al.*, 2000; Daniela *et al.*, 2004) or specific ion effects, which delay, reduces or completely inhibit seed germination (Munns, 2002; Hanselin & Eggen, 2005). In the present study the germination responses were significant among different species while, it was non-significant between the treatments.

At 15.0 dS/m salt concentration the tested species, in the order of decreasing germination percentage could be arranged as: *Linum* (85%) > *Trigonella* (62%) > *Plantago* (53%) > *Lepidium* (38%). Better seed germination performance both under low and high salinity levels exhibited by *Trigonella* is in contrast to the findings of Babu & Kumar (1979) who stated that leguminous plants are more sensitive to salinity, particularly at the germination stage. Significantly reduced germination with increasing salinity in *Linum* (Younis *et al.*, 1987; El-Nakhlaway & El-Fawal, 1989) and *Trigonella* (Ali *et al.*, 1992) have been reported. Ibrar *et al.*, (2003) and Jabeen *et al.*, (2003) reported the adverse effect of the increasing NaCl salinity on the germination of *Brassica juncea* and *Vigna mungo,* respectively. Our findings agree with them in this regard. Furthermore Anwar *et al.*, (2001); Zia & Khan (2002) also reported reduced germination under saline conditions in some other medicinal plants that also strengthen our findings.

Table 1. Mean squares of the analysis of variance for germination (%), plumule and radicle growth (mm), seedling fresh and dry weight (mg) and moisture contents (%).

Source	d.f	Germination (%)	Plumule growth (mm)	Radicle growth (mm)	Fresh weight (mg)	Dry weight (mg)	Moisture contents (%)
Concentration	6	244.905^{NS}	76.046**	38.592^{NS}	21.726^{NS}	0.289^{NS}	54812.005^{NS}
Species	3	758.226**	355.469**	408.197**	7020.238**	145.210**	1970735.677**
Error	18	98.365	13.931	24.668	26.488	0.119	38412.293
Total	27						

NS = Non-significant, * = Significant, ** = Highly significant

Table 2. Effect of various concentrations (dS/m) of NaCl on the germination (%).

Species	Concentration (dS/m)							Species mean
	Control	2.5	5.0	7.5	10.0	12.5	15.0	
Lepidium sativum	90.0	81.0	75.0	83.0	66.0	63.0	38.0	70.86^{AB}
% of Control		90.0	83.3	92.2	73.3	70.0	42.2	
Linum usitatissimum	85.0	85.0	83.0	82.0	82.0	82.0	85.0	83.43^{A}
% of Control		100.0	97.7	97.5	96.5	96.5	100.0	
Plantago ovata	71.0	61.0	63.0	55.0	69.0	38.0	53.0	58.57^{B}
% of Control		85.9	88.7	77.5	97.2	53.5	74.7	
Trigonella foenum-graecum	85.0	63.0	85.0	87.0	71.0	76.0	62.0	75.57^{A}
% of Control		71.1	100.0	102.4	83.5	89.1	72.9	
Treatment Mean	82.75	72.50	76.50	76.75	72.00	64.75	59.50	

LSD value at 0.01 alpha level for specie mean = 15.26

Means in the last column/row sharing the same letter do not differ significantly from each other at 1% level of probability

Effect on seedling growth: ANOVA for plumule growth showed highly significant differences both for the concentrations applied and the test species (Table 1). The average value for plumule growth was maximum (22.65 mm) in control condition, which decreased gradually with increasing salt concentration to 11.10 mm at 15.0 dS/m concentration. Plumule growth reduced non-significantly upto 10.0 dS/m salinity level. However, the decrease in plumule growth was significant beyond 10 dS/m. The maximum average plumule growth (26.34 mm) was recorded for *Lepidium* which was followed by *Linum* (18.67 mm), *Trigonella* (14.89 mm) and *Plantago* (9.37 mm) (Table 3).

The radicle growth varied non-significantly among the applied salinity doses while, differences between the species was highly significant (Table 1). The radicle growth showed a slight increase over control at 2.5dS/m salt concentration, which thereafter decreased. The radicle growth was maximum for *Lepidium* (24.97mm) that was closely followed by *Linum* (20.16 mm), which differed significantly from that of *Trigonella* (12.13 mm) and *Plantago* (8.086 mm) (Table 4).

Significantly reduced plumule growth with increasing salt levels in the present study agrees with the findings of Khan *et al*., (1994), Ibrar *et al*., (2003) and Jabeen *et al*., (2003) who also reported significant decline in plumule growth at 10 dS/m and higher salinity levels. Radicle growth, except certain instances, also decreased and this is in line with Ibrar *et al*., (2003) and Jabeen *et al*., (2003) who reported decline in radicle growth under saline conditions. The present findings also agree with Ali *et al*., (1992) and Khan *et al*., (1994) who reported similar results for other medicinal plants. Low salt concentrations either improved or had no pronounced effect on radicle and/or plumule growth possibly due to their nutrient like action (Hussain & Ilahi, 1992).

Effect on biomass: ANOVA indicated non-significant differences for the fresh weight among the concentration applied whereas, differences between the species were highly significant (Table 1). The average value for fresh weight slightly increased at 5.0 dS/m concentration that declined at 12.5 and 15.0 dS/m. *Trigonella* exhibited maximum average value (85.0 mg) for seedling fresh weight, that was significantly greater than *Linum* (34.29 mg), which in turn was significantly higher than *Lepidium* (20.0 mg) and *Plantago* (16.43 mg) (Table 5).

ANOVA revealed non-significant differences for dry weight of seedling among different levels of salt concentration, while highly significant differences were found between the species (Table 1). Average value for the dry weight of seedling was minimum in control (4.23 mg). It showed slight increase with each increase in concentration of the salt that reached to the maximum (5.03 mg) at the highest salt concentration. The average dry weight (Table 6) was maximum in *Trigonella* (10.91mg), followed by *Linum* (5.19 mg), *Plantago* (1.47 mg) and *Lepidium* (1.10 mg).

Generally the fresh weight of seedlings was either unaffected or got slightly decreased at higher salt concentrations. Ali *et al*., (1992) and Lyra *et al*., (1992) also reported a similar trend in the fresh weight of *Trigonella* and *Sesamum* seedlings. Reduction in fresh biomass at higher concentration might be due to poor absorption of water from the growth medium due to physiological drought (Hussain & Ilahi, 1992). Increased fresh weight under low salt concentration in *Lepidium*, *Plantago* and *Trigonella* is attributable to the development of succulence to cope with salt stress. Ilahi & Hussain (1990) also reported a similar increase in fresh weight for *Brassica campestris* and we report the same for the tested medicinal plants. The increase in the dry weight of seedlings with increasing salt concentration is in contrast to the findings of Younis *et al*., (1987) and Lyra *et al*., (1992) who reported decreased dry weight of *Linum* and *Sesamum* seedlings as a result of salt stress.

Table 3. Effect of various NaCl concentrations (dS/m) on plumule growth (mm).

Species	Concentration (dS/m)							Species mean
	Control	2.5	5.0	7.5	10.0	12.5	15.0	
Lepidium sativum	32.7	34.7	31.7	29.9	23.4	21.0	11.0	26.34^{A}
% of Control		106.1	96.9	91.4	71.6	64.2	33.6	
Linum usitatissimum	18.6	22.0	21.0	19.3	17.0	16.1	16.7	18.67^{B}
% of Control		118.3	112.9	103.8	91.4	86.6	89.8	
Plantago ovata	13.3	11.1	11.0	9.9	9.7	4.9	5.7	9.37^{C}
% of Control		83.5	82.7	74.4	72.9	36.8	42.9	
Trigonella foenum-graecum	26.0	17.0	17.3	12.3	10.4	10.2	11.0	14.89^{BC}
% of Control		65.4	66.5	47.3	40.0	39.2	42.3	
Treatment Mean	22.65^{A}	21.20^{A}	20.25^{AB}	17.85^{ABC}	15.13^{ABC}	13.05^{BC}	11.10^{C}	

LSD value at 0.01 alpha level for treatment mean = 7.597 and for specie mean = 5.743
Means in the last column/row sharing the same letter do not differ significantly from each other at 1% level of probability

Table 4. Effect of various NaCl concentrations (dS/m) on radicle growth (mm).

Species	Concentration (dS/m)							Species mean
	Control	2.5	5.0	7.5	10.0	12.5	15.0	
Lepidium sativum	38.0	35.7	24.8	25.6	20.1	19.3	11.3	24.97^{A}
% of Control		94.0	65.3	67.4	52.9	50.8	29.7	
Linum usitatissimum	17.8	24.5	25.2	21.6	14.5	14.9	22.6	20.16^{A}
% of Control		137.6	141.6	121.4	81.5	83.7	127.0	
Plantago ovata	8.2	7.0	8.2	8.6	9.7	6.5	8.4	8.09^{B}
% of Control		85.4	100.0	104.9	118.3	79.3	102.4	
Trigonella foenum-graecum	14.2	12.6	14.9	12.4	10.0	10.9	9.9	12.13^{B}
% of Control		88.7	104.9	87.3	70.4	76.8	69.7	
Treatment Mean	19.55	19.95	18.28	17.05	13.58	12.90	13.05	

LSD value at 0.01 alpha level for specie mean = 7.642
Means in the last column/row sharing the same letter do not differ significantly from each other at 1% level of probability

Table 5. Effect of various NaCl concentration (dS/m) on fresh weight /seedling (mg).

Species	Concentration (dS/m)							Species mean
	Control	2.5	5.0	7.5	10.0	12.5	15.0	
Lepidium sativum	20.0	20.0	25.0	20.0	20.0	20.0	15.0	20.00^{C}
% of Control	-	100.0	125.0	100.0	100.0	100.0	75.0	
Linum usitatissimum	40.0	40.0	30.0	35.0	30.0	30.0	35.0	34.29^{D}
% of Control	-	100.0	75.0	87.5	75.0	75.0	87.5	
Plantago ovata	15.0	15.0	15.0	20.0	15.0	20.0	15.0	16.43^{C}
% of Control	-	100.0	100.0	133.3	100.0	133.3	100.0	
Trigonella foenum-graecum	85.0	85.0	95.0	85.0	95.0	75.0	75.0	85.00^{A}
% of Control	-	100.0	111.8	100.0	111.8	88.2	88.2	
Treatment Mean	40.00	40.00	41.24	40.00	40.00	36.25	35.00	

LSD value at 0.01 alpha level for specie mean = 7.919

Means in the last column/row sharing the same letter do not differ significantly from each other at 1% level of probability

Table 6. Effect of various concentration of NaCl (dS/m) on dry weight /seedling (mg).

Species	Concentration (dS/m)							Species mean
	Control	2.5	5.0	7.5	10.0	12.5	15.0	
Lepidium sativum	1.1	1.1	1.1	1.1	1.0	1.1	1.2	1.10^{C}
% of Control		102.8	99.1	105.7	94.3	107.6	111.3	
Linum usitatissimum	4.9	4.8	5.0	5.3	5.4	5.2	5.7	5.19^{B}
% of Control		98.6	101.6	108.6	109.4	107.2	117.2	
Plantago ovata	1.4	1.4	1.4	1.4	1.6	1.5	1.6	1.47^{C}
% of Control		101.5	101.5	100.7	113.8	108.7	115.2	
Trigonella foenum-graecum	9.5	10.4	11.0	11.2	11.3	11.4	11.6	10.91^{A}
% of Control		109.1	116.1	117.7	119.5	120.4	122.4	
Treatment Mean	4.23	4.43	4.63	4.75	4.83	4.80	5.03	

LSD value at 0.01 alpha level for specie mean = 0.5308

Means in the last column/row sharing the same letter do not differ significantly from each other at 1% level of probability

Table 7. Effect of various concentrations of NaCl (dS/m) on seedling moisture contents (%).

Species	Concentration (dS/m)							Species mean
	Control	2.5	5.0	7.5	10.0	12.5	15.0	
Lepidium sativum	1786.8	1734.9	2281.0	1685.7	1900.0	1654.4	1171.2	1744.86[A]
% of Control		97.1	127.7	94.3	106.3	92.6	65.6	
Linum usitatissimum	718.0	729.9	503.6	559.1	367.3	572.5	510.8	565.89[C]
% of Control		101.7	70.1	77.9	51.2	65.8	71.2	
Plantago ovata	987.0	971.4	971.4	1338.9	855.4	1233.3	843.4	1028.69[B]
% of Control		98.4	98.4	135.7	86.7	125.0	85.5	
Trigonella foenum-graecum	795.7	721.3	762.1	661.0	737.7	556.2	545.4	682.77[C]
% of Control		90.6	95.8	83.1	92.7	69.9	68.6	
Treatment Mean	1071.88	1039.38	1129.53	1061.18	965.10	1004.10	767.70	

LSD value at 0.01 alpha level for specie mean = 301.5

Means in the last column/row sharing the same letter do not differ significantly from each other at 1% level of probability

Effect on moisture content: Statistically, moisture contents also varied non-significantly among the various concentration of salt applied. However, highly significant differences were observed among test species (Table 1). The moisture contents were generally similar or slightly more than control in *Lepidium* except at 15 dS/m, *Plantago* and *Trigonella*. In *Linum*, moisture contents declined in all the treatments except 2.5 dS/m. The average moisture contents were maximum (1129.53%) at 5.0 dS/m while, minimum (767.70%) at 15.0 dS/m level of salt (Table 7). The maximum average value for moisture content obtained for *Lepidium* (1744.86%) was significantly different from *Plantago* (1028.69%), which in turn varied significantly from *Trigonella* (682.77%) and *Linum* (565.89%).

Moisture contents of seedlings play important role in various physiological processes including growth. In the present study moisture contents of seedling increased or decreased inconsistently under different salt concentrations. The increased moisture contents at some instances was due to the development of succulence to minimise the adverse effect of salts (Ilahi & Hussain, 1990). This also explains why the fresh weight enhanced under salt treatment. Shadded & Zaidan (1989) recorded reduced water contents with increased salt stress in *Trigonella* while Ibrar & Hussain (2003) reported enhanced root moisture contents with increasing salinity level in *Medicago polymorpha*.

This preliminary laboratory study suggests that the tested medicinal species could be grown on marginally saline habitats due to their tolerance to moderate salinity at germination and seedling stage. However, further study is needed to test their salt tolerance under field conditions to assess the possibility of cultivation of medicinal plants on otherwise unproductive lands.

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