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Research Article

EFFECTS OF SEED PRIMING ON GERMINATION TRAITS OF *NIGELLA SATIVA* UNDER SALINE CONDITIONS

***Maryam Najar and Saeid Bakhtiari**
Department of Agronomy, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran
**Author for Correspondence*

ABSTRACT
Two separate experiments were conducted to investigate the effect of seed priming on germination and vegetative characteristics of *Nigella sativa* under saline condition. Two factorial experiments based on completely randomized design with three replications carried out in laboratory and greenhouse at2014. Factors were five priming levels (control, hydro-priming with distilled water, osmo-priming with 25 mM solution of NaCl, 0.5 % solution of $CaSo_4$and 0.5% solution of KNO_3) and four salinity levels (0, 25, 50 and 75 mM solution of NaCl).
Germination rate and percentage, radical and hypocotyls length and dry weight measured in laboratory phase. Germination rate and percentage root and shoot length and dry weight, leaf area, leaf number and weight were measured in green house phase. Results showed germination rate and percentage were both decreased by salinity. The highest germination rate and percentage belonged to seeds which treated with distilled water and NaCl solution in laboratory and greenhouse respectively. The highest root and shoot dry weight produced by hydro-priming treatment. The highest shoot length and leaf area observed for seedlings which grew from NaCl treated seed.

Keywords: *Germination Percentage, Germination Rate, Root and Shoot Dry Weight, Leaf Area*

INTRODUCTION
Soil salinity affects a large and increasing amount of arable land worldwide and agronomic solutions to increasing salt tolerance are urgently needed. Germination is a critical stage of the plant cycle and improved tolerance of high salinity could improve the stability of plant production and produce a stable seedling establishment (Jarami, 2009; Rauf *et al.,* 2007). Effect of salinity on shoot growth rate, shoot and root length and seed nutrition consuming rate by seedling are reported (Soltani *et al.,* 2001; Iran Nezhad *et al.,* 2009). Seed germination affects by salinity via low water potential, ion toxicity of chloride, sodium or other ions and lack of nourishment ions such as potassium and calcium (Khan and Golzar, 2003).
Priming is technique which enhances germination characteristics and seedling establishment. During priming, seeds are hydrated to a level below that needed for radical emergence, allowing pre-germination metabolism to proceed (Mc Donald, 2000).
Nigella sativa is a pharmaceutical plant belongs to family Ranunculaceae. Seeds of nigella contain 30-40 percent oil, 20 percent protein, 7.5 percent humidity and about 0.5-1.5 percent essential oils. The seeds have a complex chemical structure with many different chemical constituents. The main constituents of nigella are alkaloids, fixed and essential oils. Thymoquinone is the most active constituents of the seeds, representing 18.4–24% of the essential oil (Al-Saleh *et al.,* 2006). Nigell seed have been used in traditional medicine, either alone or mixed with, for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney, bladder and liver function, circulatory and immune system support, and for general overall well-being (Baser *et al.,* 1986; Handa, 1998; Malhotra, 2006).
Fathi *et al.,* (1391), investigated the effect of seed priming on germination rate and percentage of nigella at saline condition. Results showed that germination rate and percentage affected by salinity, priming and interaction between then. In all salinity levels, the highest germination rate gained by applying KNO3 and/or distilled water plus with gibberellic acid. Moosavi *et al.,* (2013) investigated the effect of salt stress on germination and early seedling growth of *Nigella sativa*. Results showed that the application of

8 ds m^{-1} NaCl solution resulted in the loss of seedling length, seedling weight, germination percentage, and germination rate and seed viability index.

MATERIALS AND METHODS

The study carried out as two separate experiment sat 2014. Laboratory and greenhouse experiments carried out at Azad university and technique and profession school of Neyshabour respectively. Two factorial experiments base on completely randomized design with three replications was carried out. Factors were five priming levels (control, hydro-priming with distilled water, osmo-priming with 25 mM solution of NaCl, 0.5 % solution of $CaSo_4$ and 0.5% solution of KNO_3) and four salinity levels (0, 25, 50 and 75 mM solution of NaCl). In each priming treatment, 350 seeds of Nigella seeds soaked in 1000 cc bakers of prepared solution. Beakers placed in germinators with 25±1°c temperature for 24 hours. Then seeds rinsed with tap water three times. In laboratory phase, 100 seeds of each treatment placed in petri dishes on a layer of filter paper. Petri dishes were irrigated with different saline solutions of NaCl. Seed germination was recorded daily up to day 14 after the start of the experiment. A seed was considered germinated when the radical emerged by about 2 mm in length. Germination percentage and rate calculated using the following equitation:

$$germination\,(\%) = \frac{number\ of\ germinated\ seed}{total\ seeds\ number} \times 100$$

$$GR = \sum \frac{ni}{di}$$

While ni is the number of germinated seeds per day and di is the day of counting (Ellis and Roberts, 1981). Radical and hypocotyls length of 5 random samples measured by a ruler. Then samples placed in an oven for 48 hours at 70°c. Dry weight measured by a digital scale. The same methods was taken for greenhouse phase while 10 treated seeds planted in 30×30 cm pots in each priming level and irrigated with NaCl solution in order to performing greenhouse study. The data of experiment analyzed by ANOVA using the SAS statistical package and significance of differences between means was conducted using Duncan's multiple range test at P=0.01.

RESULTS AND DISCUSSION

Laboratory

Germination percentage significantly affected by salinity (p<0.01), priming (p<0.01) and interaction between them (p<0.05) (table 1).

Table 1: Analysis of variance of different traits at laboratory

Source of variation	Degree of freedom	Mean squares G%		G.R		Radical length		Hypocotyls length		Radical dry weight		Hypocotyls dry weight	
Salinity (s)	3	1290	**	102	**	5.59	**	5.08	**	6.64	**	5.03	**
Priming (p)	4	684	**	85.79	**	1.12	**	0.91	**	6.35	**	3.25	**
S*P	12	153	**	6.99	*	0.074	**	0.093	**	2.94	**	1.39	*
Error	60	59.85		3.41		0.015		0.016		2.39		5.32	
c.v %		10.1		9.76		11.36		14.31		16.05		14.87	

** and ** significant at 5 and 1 percent probability levels*

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Germination rate decreased by higher salinity level. NaCl priming increased the germination rate in all salinity levels (figure 1). The same results seen for germination rate (GR), while GR affected by interaction between salinity and priming ($p<0.05$) results showed that GR decreased by applying KNO_3 as priming solution (table3). Lower germination percentage and rate of seeds treated by KNO_3, could be the results of high negative water potential of the solution compare with other solutions. Different response of plant seeds to various priming solutions also is due to physiological differences.

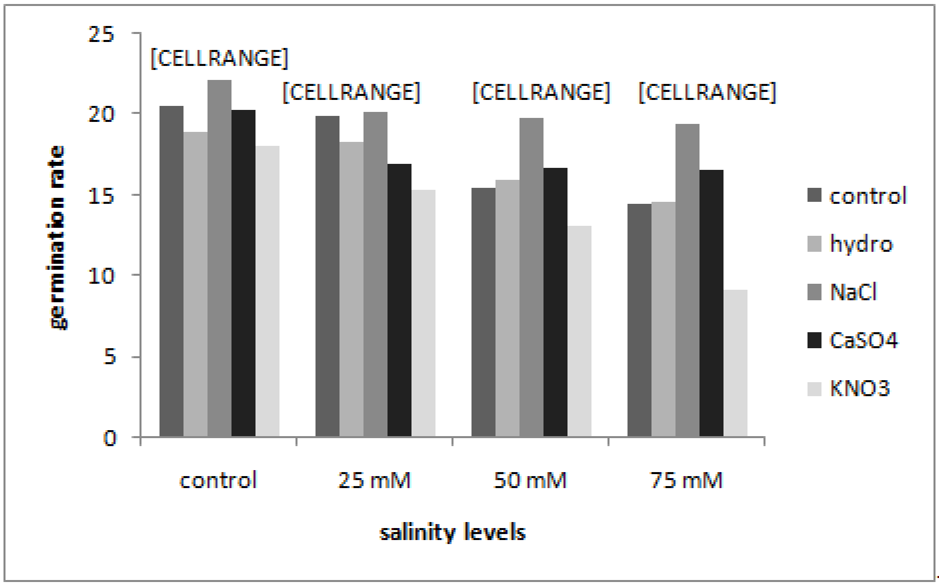

Figure 1: Germination rate as affected by interaction between salinity and priming

Demir *et al.*, (2006), reported that seed germination affect by salinity due to low water absorption because of high negative water potential, and the toxic effect of ions within the cell.
Seed germination accelerate by priming via enhancing enzymatic activities of alpha amylase, increasing energy-bearing molecules such as ATP, improving RNA and DNA synthesis and enhancing mitochondria efficiency (Penalosa and Eira, 1993).
Radical and hypocotyls length and dry weight significantly affected by salinity, priming and interaction between them (table 1). Radical and hypocotyls dry weight decreased in saline conditions. Higher NaCl levels resulted in shorter seedling length. Length improved by hydro-priming in control treatment as well as all salinity levels (table 3). Hypocotyls length decreased 48.38 percent by applying 75 mM NaCl while Radical length decreased more than 61 percent.
In response to salt stress, the abscisic acid (ABA) content increases, and gibberellic acid (GA) and decreases (Xu *et al.*, 2011).GA promotes radical and hypocotylslength during seed germination, and promotes early seedling development while ABA suppress germination (Gallardo *et al.*, 2002).
The interaction of seed priming and salinity level significantly ($P < 0.01$) affected the radical and hypocotyls dry weights (table 1). As salinity level increase, the reduction in dry matter increased proportionally. But the inhibitory effect was more increased in weights recorded from unprimed seeds in relation to primed seeds (table 3). The toxic effect of sodium at high salt levels and physical damage to roots decreased their ability to absorb water and nutrient which caused strong reduction in photosynthesis, enzymatic process and protein synthesis. This resulted in limited growth and dry matter accumulation in plant organs (Akram *et al.*, 2010).
Higher cell deviation rate in root cap caused by seed priming, beside higher water and nutritional element uptake, results in higher root growth and development (Afzal *et al.*, 2009).

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Table 2: analysis of variance of different traits at greenhouse

		Mean squares								
Source of variation	**Degree of freedom**	**G%**	**G.R**	**Root length**	**Shoot length**	**Root dry weight**	**Shoot dry weight**	**Leaf dry weight**	**Leaf area**	**Leaf number**
Salinity (s)	3	5048 **	4.43 **	148 **	76.32 **	0.01 **	5.03 **	0.05 **	47.75 **	55.26 **
Priming (p)	4	319 **	0.41 **	12.21 **	53.33 **	0.008 **	3.25 **	0.02 **	7.95 **	13.15 **
S*P	12	94.72 **	0.08 **	0.61	0.74 *	0.0004 **	1.39 *	0.002 **	0.45 *	0.37
Error	60	28.33	0.02	0.37	0.34	7.66	5.32	0.0001 **	0.2	0.22
c.v %		9.38	12.3	12.3	13.11	16.2	12.63	14.63	9.6	7.32

** and ** significant at 5 and 1 percent probability levels*

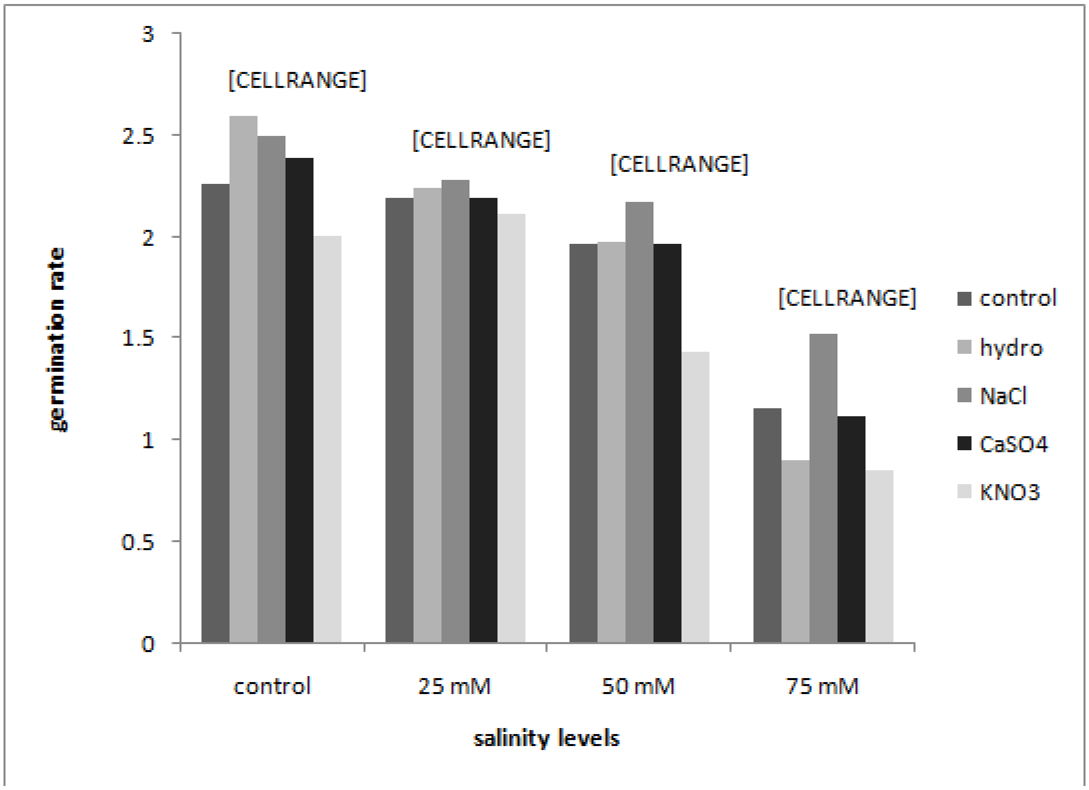

Figure 2: germination rate as affected by interaction between salinity and priming

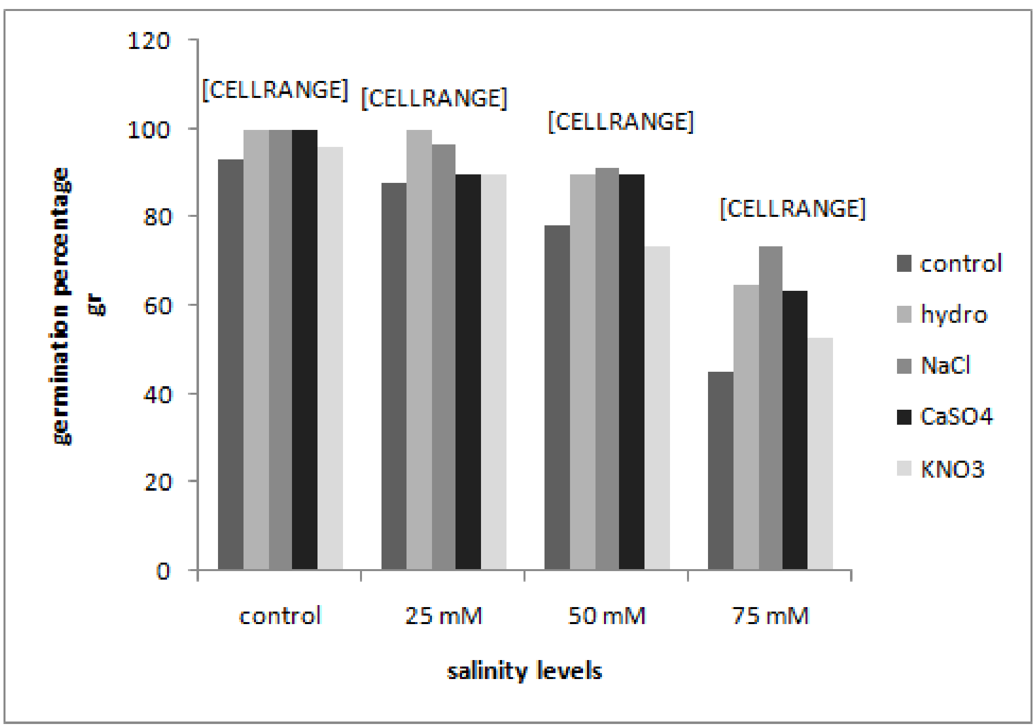

Figure 3: germination percentage as affected by interaction between salinity and priming

Table 3: comparison of means using Duncan's test at laboratory

treatment	G%		GR (germinated seeds/day)		radicel length (mm)		hypocotyle length (mm)		radicle dry weight (gr)		hypocotyle dry weigth (gr)	
S1	94.40	a	19.98	a	2.30	a	2.52	a	0.006	a	0.006	a
S2	89.10	ab	18.10	ab	1.47	ab	2.07	b	0.003	b	0.005	b
S3	82.45	ab	16.16	bc	1.15	b	1.57	c	0.002	c	0.003	c
S4	75.90	b	14.79	c	0.89	c	1.19	d	0.001	c	0.002	c
P1	85.88	ab	17.55	ab	0.11	c	1.64	b	0.003	bc	0.003	b
P2	85.38	ab	16.91	bc	0.23	a	2.31	a	0.004	a	0.005	a
P3	95.25	a	20.36	a	0.20	b	1.67	b	0.003	ab	0.004	ab
P4	83.88	ab	17.60	ab	0.10	cd	1.84	b	0.002	c	0.004	ab
P5	76.94	b	13.87	c	0.08	d	1.73	b	0.003	bc	0.004	ab
S1*P1	95.75	a	20.52	ab	2.51	b	2.07	ef	0.005	d	0.005	b-e
S1*P2	92.50	abc	18.88	b-e	3.02	a	3.02	a	0.009	a	0.008	a
S1*P3	97.00	a	22.12	a	1.87	d	2.31	cd	0.007	b	0.006	b
S1*P4	92.75	abc	20.32	ab	2.18	c	2.78	b	0.004	de	0.006	b-d
S1*P5	94.00	abc	18.08	b-f	1.90	d	2.45	c	0.005	c	0.006	bc
S2*P1	94.75	ab	19.84	abc	1.51	ef	1.80	gh	0.003	e	0.005	c-f
S2*P2	92.00	abc	18.27	b-f	1.83	d	2.49	c	0.003	e	0.006	bc
S2*P3	95.50	a	20.15	ab	1.31	fg	1.93	fg	0.004	d	0.004	e-g
S1*P4	81.50	cd	16.97	c-g	1.31	fg	2.10	d-f	0.002	fg	0.004	e-h
S2*P5	81.75	cd	15.24	f-h	1.40	f	2.06	ef	0.003	ef	0.005	d-f
S3*P1	78.25	d	15.41	f-h	1.29	fg	1.64	hi	0.002	g-i	0.003	g-i
S3*P2	82.00	bcd	15.98	e-h	1.70	de	2.17	de	0.002	gh	0.003	g-i
S3*P3	94.25	abc	19.78	a-c	0.82	ijk	1.21	jk	0.002	g-i	0.003	i-k
S3*P4	82.00	bcd	16.61	d-g	1.02	hi	1.43	ij	0.002	g-i	0.003	h-j
S3*P5	75.75	d	13.04	h	0.92	ij	1.42	ij	0.002	gh	0.004	h-j
S4*P1	74.75	d	14.43	gh	0.98	hij	1.07	k	0.001	hi	0.002	k
S4*P2	75.00	d	14.53	gh	1.15	gh	1.58	hi	0.002	g-i	0.002	i-k
S4*P3	94.25	abc	19.39	a-d	0.78	jk	1.23	jk	0.001	i	0.002	jk
S4*P4	79.25	d	16.51	d-g	0.95	hij	1.07	k	0.001	i	0.003	i-k
S4*P5	56.25	e	9.10	i	0.59	k	1.07	k	0.001	i	0.002	jk

Harris *et al.*, (2000) reported that faster emergence of primed seeds, could result in better resource capture and contribute to higher plant growth.

Greenhouse

Germination rate and percentage affected by salinity, priming and interaction between them ($p<0.01$) (table 2). The increase in salt solution in culture medium resulted a significant decrease in germination rate ad percentage. However, the decrease was more significant for non-primed seeds than NaCl and KCl seed priming (figure 2, 3).

Table 4: comparison of means using Duncan's test at greenhouse

treatment	G%		GR (germinated seeds/day)		root length (mm)		shoot length (mm)		root dry weight (gr)		shoot dry weigth (gr)		leaf dry weigth (gr)		leaf number		leaf area (cm2)	
S1	2.31	a	98.67	a	18.49	a	18.11	a	0.14	a	0.25	a	0.23	a	12.29	a	7.36	a
S2	2.20	a	96.00	ab	16.59	b	15.98	b	0.10	b	0.20	b	0.17	b	11.24	b	5.78	b
S3	1.90	b	88.00	b	13.84	c	15.03	b	0.07	c	0.15	c	0.12	c	9.51	c	4.59	c
S4	1.11	c	58.67	c	11.31	d	12.67	c	0.06	c	0.13	c	0.09	d	7.93	d	3.16	d
P1	1.85	ab	85.00	ab	14.01	c	14.91	c	0.08	b	0.15	cd	0.12	c	9.93	b	4.80	cd
P2	1.93	a	85.00	ab	15.43	b	16.08	b	0.09	b	0.21	b	0.17	b	10.98	a	5.39	bc
P3	2.12	a	91.67	a	16.61	a	18.78	a	0.13	a	0.25	a	0.22	a	11.66	a	6.22	a
P4	1.92	a	87.50	a	14.78	bc	13.96	cd	0.09	b	0.18	bc	0.13	c	9.46	b	5.62	ab
P5	1.60	b	77.50	b	14.46	bc	13.50	d	0.06	c	0.13	d	0.13	c	9.18	b	4.08	d
S1*P1	2.26	de	90.00	a	16.94	cd	17.33	de	0.12	c	0.25	bcde	0.17	cd	11.47	de	6.62	c
S1*P2	2.60	a	100.00	a	19.53	b	18.47	bc	0.15	b	0.29	a	0.31	a	12.80	b	7.56	b
S1*P3	2.49	ab	100.00	a	20.63	a	22.50	a	0.20	a	0.30	a	0.30	a	13.73	a	8.51	a
S1*P4	2.38	a-c	100.00	a	17.68	c	16.37	ef	0.12	c	0.25	bcd	0.19	c	11.73	cde	7.60	b
S1*P5	2.00	de	96.00	a	17.64	c	15.87	f	0.09	de	0.17	gh	0.19	c	11.70	cde	6.53	c

S2*P1	2.19	c-e	93.33	a	16.00	de	15.90	f	0.10	d	0.15	hi	0.13	ef	11.13	ef	5.54	d
S2*P2	2.24	b-e	100.00	a	16.47	d	16.47	ef	0.10	d	0.25	bc	0.17	cd	12.47	bc	6.11	cd
S2*P3	2.28	b-d	96.67	a	17.73	c	19.33	b	0.13	c	0.29	ab	0.25	b	12.28	bcd	6.29	cd
S1*P4	2.19	c-e	90.00	a	16.47	d	14.40	g	0.09	de	0.18	gh	0.15	de	10.33	fg	6.18	cd
S2*P5	2.11	c-e	90.00	a	16.30	d	13.80	g	0.06	fg	0.15	hi	0.13	ef	10.00	gh	4.77	e
S3*P1	1.97	e	78.00	b	13.00	g	14.00	g	0.07	f	0.10	j	0.09	gh	9.37	h	4.22	ef
S3*P2	1.98	e	90.00	a	14.07	fg	16.00	f	0.08	ef	0.16	hi	0.11	fg	10.40	fg	4.73	e
S3*P3	2.17	c-e	91.00	a	15.07	ef	17.87	cd	0.10	d	0.23	cdef	0.17	c	11.07	ef	6.25	cd
S3*P4	1.97	e	90.00	a	13.70	g	13.87	g	0.07	f	0.16	ghi	0.12	f	8.57	i	4.54	ef
S3*P5	1.43	f	73.33	bc	13.37	g	13.40	ghi	0.04	h	0.12	ij	0.11	fg	8.13	i	3.19	gh
S4*P1	1.16	g	45.00	e	10.10	i	12.40	h j	0.05	gh	0.08	j	0.07	h	7.77	ij	2.83	h
S4*P2	0.90	g	65.00	cd	11.63	h	13.40	gh	0.05	gh	0.12	ij	0.08	h	8.23	i	3.14	gh
S4*P3	1.52	f	73.33	bc	13.00	g	15.40	f	0.10	d	0.21	d fg	0.15	de	9.57	gh	3.83	fg
S4*P4	1.12	g	63.33	d	11.27	hi	11.20	k	0.06	fg	0.12	ij	0.08	h	7.20	j	4.17	ef
S4*P5	0.85	g	53.00	e	10.53	i	10.93	k	0.04	h	0.10	j	0.08	h	6.90	j	1.84	i

Root and shoot length affected by salinity ($p<0.01$) and priming ($p<0.01$). Shoot affected by interaction between treatments ($p<0.05$) but root did not affect significantly (table 2). Shoot length decreased by salinity, this reduction was significantly higher for non-primed seeds, compared to primed seeds (table 4). Increase of root length in primed seeds as compared to the unprimed could be a result of embryo cell wall extensibility.

Root length decreased by salinity. Between priming solutions (figure 4), the highest root length produced by NaCl solution (figure 5).

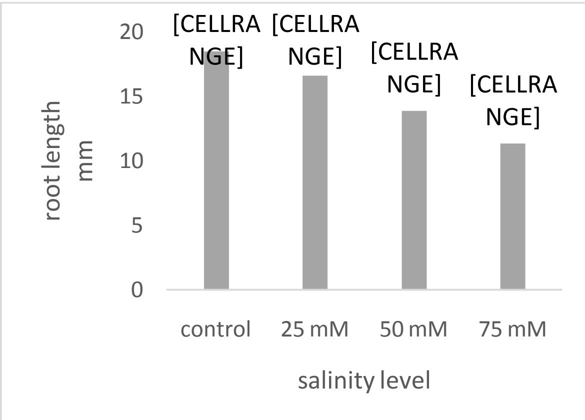

Figure 4: root length as affected by salinity levels

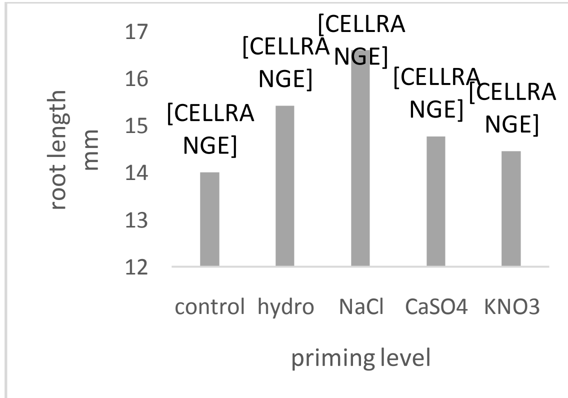

Figure 5: root length as affected by priming levels

Leaves number per plant, affected by salinity and priming ($p<0.01$) (table 2). Levees number reduced by salinity (table 4). Hydro-priming and osmo-priming with NaCl resulted in higher leaves number per plant (table 4).

Leaf area affected by interaction between salinity and priming (table 2). Leaf area decreased by increasing salinity stress. However, leaf area production by primed seeds was higher than those of nonprime seeds (table 4). Leaf stomata close during salinity stress due to higher content of ABA which results in lower water evapotranspiration and suppressing leaf growth (Wang *et al.*, 2007).

Shoot, root and leaf dry weight affected by salinity, priming and interaction between them ($p<0.01$) (table 2). Dry weight decreased by salinity stress. A seed which primed with NaCl solution and distilled water produced root, shoot and leaves with higher dry weight (table 4). Reduction in plant growth is an adoptability aspect at stress condition. Nutritional elements consume in plant maintenance instead of plant growth and development (Khalid, 2006).

Conclusion

Results showed that Nigella is sensitive to salinity during germination stage. Higher salinity results in lower germination rate and percentage and affect seedling characteristics. Germination and seedling traits improved in primed seeds in both control and stress conditions. Between different priming treatments, hydro-priming and applying NaCl solutions showed proper results in most of measured treatments. The most remarkable effect of seed priming seen for root and shoot length and seedling weight due to accelerated germination. Salinity resulted in lower leaf number and leaf area per plant which consequently decrease photo synthetically active organs and dry matter accumulation in nigella plants.

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