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Research Article

EFFECT OF PRIMING ON SEED GERMINATION AND SEEDLING CHARACTERISTICS OF *NIGELLA SATIVA* AT DROUGHT CONDITIONS

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ABSTRACT
In order to investigate the effects of seed priming on germination indices and seedling characteristics, two separate experiments were conducted at Neyshabour during 2014 growing season. Two factorial experiments base on completely randomized design with three replications carried out at laboratory and greenhouse separately. Treatment were include six priming levels (applying 0, -3, -6, -9 and-12 bar solutions of PEG6000 and hydro-priming with distilled water) and three drought levels. Drought treatment induced using PEG6000 in three concentrations (0, 7.5 and 15 bar) at laboratory. In greenhouse drought induced by irrigating100, 50 and 25 percent of field capacity. Germination percentage and rate, radicel and hypocotyl length and dry weight, root and shoot length, root and shoot and leaves dry weight leaf number and leaf area per plant was measured. Results showed that *Nigella sativa* germination was sensitive to drought and higher drought intensity resulted in lower germination percentage and rate. Other seedling traits injured by drought too. Seed priming diminished negative effects of drought and higher germination percentage and rate observed in primed seeds. Drought resulted in lower green area in each plant by reducing leaf number and leaf area, thus photosynthesis decreased. Total dry matter aggregation decreased due to low photosynthesis capacity in each plant.

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

INTRODUCTION
Drought is one of the most serious worldwide problems in crop production. More than 45% of agricultural lands are permanently exposed to drought stress (Ashraf and Foolad, 2007). Germination is a critical stage of the plant life. Water availability of the soil is considered one of the principal causes of low germination in seeds (Jajarmi, 2009).

Resistance against drought stress during the germination results in a stable plant stand. In crop production, stand establishment determines plant density, uniformity and management options (Cheng and Bradford, 1999). Seed viability and vigor can have an influence on the establishment and yield of crops. Also a vigorous early seedling growth has been shown to be associated with higher yields (Harris *et al.*, 2000; Mussa *et al.*, 1999).

Seed priming is a technique of seed enhancement which improves germination or seedling growth and rate or uniformity of the seedling establishment (Taylor *et al.*, 1998). Seed quality of most species is improved by preconditioning. Seed priming had also been reported to result in better seedling growth under water deficit stress conditions (Kaur *et al.*, 2002). It has long been known that wetting treatments can improve seed germination. In recent years, an increasing range of chemical seed treatments have also become available (Halmer 2000). Treated seeds are usually re-dried to primary moisture before use, but they would exhibit rapid germination when reimbibed under normal or stress conditions (Ashraf and Foolad 2005).

Nigella sativa L. known as black seed or black cumin, is an annual herb from family of Ranunculaceae. The seeds of nigella have been used in the Southeast Asia, Middle and Far East as a natural remedy to treat many diseases, including asthma, hypertension, diabetes, hypercholesterolemia, inflammation, arthritis, tumor, gastrointestinal disturbances and gynecological disorders for over 2000 years (Ali and Blunden, 2003, El-Din *et al.*, 2006, Ramadan, 2007).

Kabiri *et al.*, (2012) investigated the effect of drought stress and its interaction with salicylic acid (SA) on *Nigella sativa* germination and seedling growth. Results indicated that germination rate and percentage, seed vigor index, root and shoot length and dry weight decreased by drought stress. The seeds treated by SA produced higher root and shoot length and dry weight. Burnett *et al*., (2005) investigated the effect of different osmotic stress obtained with polyethylene glycol6000 solutions on the germination of marigold seeds.

The rate of seed germination and the final germination percentage decreased by lower amount of water absorbance. Stephanie *et al.,* (2005) reported the same results in *Salvia solendens* seeds.

MATERIALS AND METHODS

Two separate factorial experiments base on completely randomized design with three replications carried out in laboratory and greenhouse at 2014. Seed germination traits first studied in laboratory of Azad university of Neyshabour and then in the greenhouse of technique and profession school of Neyshabour.

Factors were include six priming levels (applying 0, -3, -6, -9 and-12 bar solutions of PEG6000) in both laboraty and greenhouse and three drought levels. Drought treatment induced using PEG6000 in three concentrations (zero, 7.5 and 15 bar) at laboratory. In greenhouse drought induced by irrigating100, 50 and 25 percent of field capacity.

According to the method presented by (Michel and Kaufmann, 1973) solutions with different osmotic potential were prepared for priming and drought stress treatments. For each treatment 350 Nigella seed were soaked in 1000 cc of priming solution. Solution containers placed in germinators with 25±1°c temperature for 24 hours. Then seeds washed with tap water three times. Three replications of 100 seeds in each priming treatment placed within 90mm petri dishes on filter papers.

During the germinating test 5ml of drought solutions or distilled water added to each petri dishes when filter papers grew waterless. Germinated seeds (with a 1-2 mm radicel) counted every 24 hours for 14 days. Germination percentage

(GP) was calculated applying Scott *et al.,* (1984):

$$GP = \frac{total\ germinated\ seeds\ (when\ no\ further\ germination\ occured)}{total\ number\ of\ seeds} \times 100$$

Germination rate (GR) was calculated according to Maguire's (1962):

$$GR = \frac{E_1}{N_1} + \frac{E_2}{N_2} + \cdots + \frac{E_{Nn}}{N_n}$$

Where En is the number of germinated seeds observed in the nth daily counting and Nn is the number of days after the seeds were put to germinate in the nth counting. Radicel and hypocotyl length of five random samples were measured after the 14th day.

Thus samples oven dried at 70°C for 48 hours and weighted with digital scale. The same methods applied for greenhouse stage while 10 seeds of each priming treatment planted in 30×30 cm pots irrigated with 100, 50 and 25 percent of field capacity water amounts. Data analyzed using SAS software. Comparison between means was conducted using Duncan's multiple range test at 0.01 significant levels.

RESULTS AND DISCUSSIONS

Laboratory

Analysis of variance showed that germination rate and percentage affect by drought, priming and interaction between them ($p<0.01$) (table 1).

Higher drought stress resulted in lower germination rate and percentage. Reduction in the seeds water content due to low media water potential decreases the activity of hydrolytic enzymes such as alpha-amylase, proteases and lipases which are responsible in hydrolyzing cotyledons and thus results in lower seed germination rate (Dahal *et al*., 1996; Zayed and Zeid, 1998).

Table 1: Analysis of variance of different traits at laboratory

Source of variation	Degree of freedom	Mean squares					
		G%	G.R	Radicel length	Hypocotyl length	Radicel dry weight	Hypocotyl dry weigth
Drought (D)	2	10087 **	875 **	3.77 **	5.37 **	3.34 **	2.46 **
Priming (p)	5	1204 **	152 **	1.70 **	3.48 **	6.27 **	5.71 **
D*P	10	234 **	43.41 **	0.21 **	0.17 **	3.96	5.56 **
Error	36	3.32	1.87	0.01	0.04	4.51	9.74
c.v %		9.1	11.38	12. 13	12.26	11.64	13.68

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

GR and G% of primed seeds lower affected by drought (table 3). In control treatment, hydro-priming results in higher GR compared with none primed seeds (figure 1). Seed priming causes metabolic alterations in seeds include changes in cell cycle process (Castro *et al.*, 2000), accelerate endosperm analysis by higher hydrolase activities (Groot *et al.*, 1988; Bradford *et al.*, 2000) and higher mobilization of storage proteins (Job *et al.*, 2000).

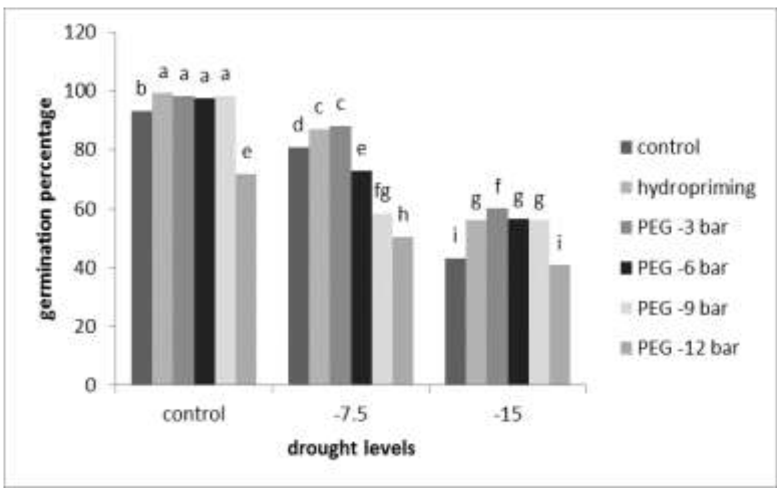

Figure 1: germination percentage as affected by interaction between drought and priming

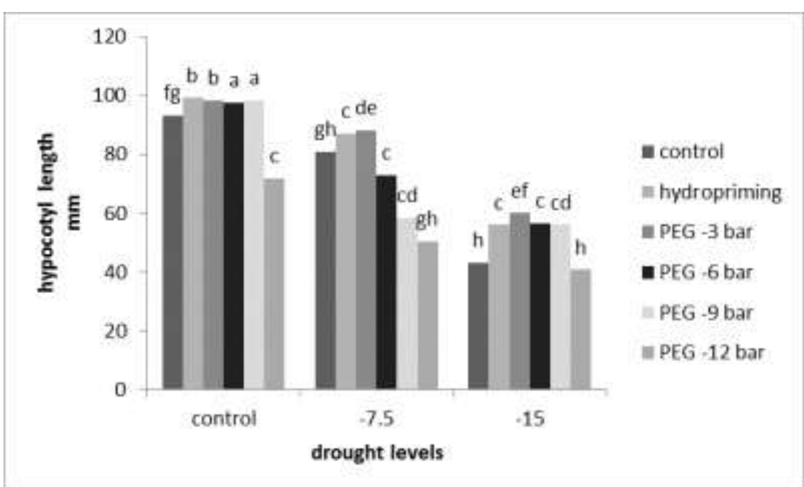

Figure 2: hypocotyl length as affected by interaction between drought and priming

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Radicel and hypocotyl length and hypocotyl weight affected by drought, priming and interaction between them ($p<0.01$) (table 1). Radicel weight affected by drought ($p<0.01$) and priming ($p<0.01$) but not but interaction between treatments (table 1). Radicel and hypocotyl length and dry weight significantly decreased by drought. Growth parameters increased in primed seeds compared with non-primed seeds (table 3). The highest hypocotyl length produced by seeds which treated with -6 and -9 bar solutions of PEG6000 at sin-stress condition (figure 2).

The highest radicel length produced by hydro-priming followed by seeds treated with -3 and -6 bar solutions of PEG6000 in sin-stress condition (table 3). Radicel length decreases by drought due to lower cell deviation rate and development (Shakirova *et al*., 2003).

Radicel emergence accelerate by priming via higher enzymatic activities, protein synthesis and ATP production (Parera and Cantliffe, 1992).

There was no significant difference between control and -7.5 bar drought level in respect of radicel dry weight). Radicel dry weight reduced by -15 bar (table 3). The highest radicel dry weight produced by seeds which treated with distilled water (table 3). Primed seeds has better efficiency in water absorption from growing media, that’s why metabolic activities in seed during germination process commence much earlier than those of unprimed seeds (Hopper *et al.*, 1979).

Greenhouse

Results of germination test showed that G% and GR significantly affect by drought, priming and interaction between them ($p<0.01$) (table 2). G% and GR decreased by drought while higher germination rate and percentage was obtained from primed seeds compared to control seeds (table 4). Needed time for seed germination is shorten by priming due to faster water uptake and earlier initiation of metabolism processes (Kaya *et al.*, 2006). Falleri *et al.,* (2004) reported that direct effects of slower decomposition of endosperm or slower transition of decomposed materials to seedlings reduce the percentage of germination in the water stress conditions. Gastel *et al.*, (1996) found that during water shortage periods, cell walls could not absorb water easily and conduct it to endosperm and thus germination occur later.

Root and shoot length significantly affected by drought level ($p<0.01$) and priming ($p<0.05$ for shoot and $p<0.01$ for root) (table 2). The plants of primed seeds attained more height than those of non-primed seeds (fig 3). The enhanced shoot length in primed seeds may be due to the improved and faster plant emergence in primed seeds which created competition among plants for light and resulted in taller plants (Arif, 2005).

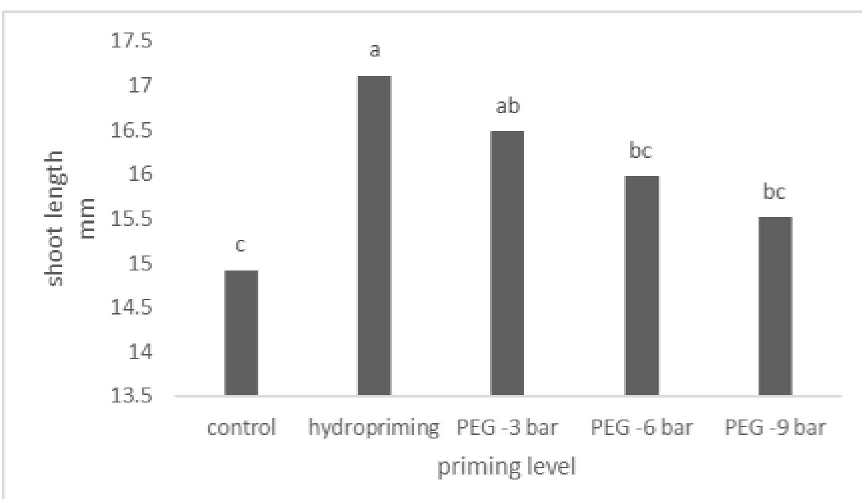

Figure 3: Shoot length as affected by priming levels

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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 weigth	Leaf dry weight	Leaf area	Leaf number
Drougth (D)	3	1516**	1.29**	8.32**	58.48**	0.001**	0.002**	7.43**	47.75**	8.02**
Priming (p)	4	453**	0.78**	16.98**	5.87*	0.001**	0.001**	1.33**	7.95	4.46**
D*P	12	76.66*	0.17**	1.62	1.69	4.022	0.0002*	0.28**	0.45	0.61
Error	60	29.62	0.05	1.06	1.77	5.15	0.0001	0.54**	0.2	0.49
c.v %		11.07	9.76	12.3	12.55	15.11	14.34	13.36	9.6	6.69

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

Table 3: Comparison of means using Duncan multiple test at laboratory

treatment	G%		GR (germinated seeds/day)		radicel length (mm)		hypocotyle length (mm)		radicle dry weight (gr)		hypocotyle dry weigth (gr)	
D1	93.04	a	18.66	a	3.05	a	2.21	a	0.002	a	0.005	a
D2	72.96	b	10.02	b	2.61	b	1.35	b	0.002	a	0.003	b
D3	52.04	c	7.04	c	2.13	c	1.20	b	0.002	b	0.003	c
P1	72.42	c	11.73	bc	2.22	c	0.74	c	0.002	c	0.004	b
P2	80.75	a	17.73	a	2.82	b	2.01	a	0.002	a	0.005	a
P3	82.08	a	13.43	b	2.98	a	1.58	b	0.002	ab	0.003	c
P4	75.58	b	10.53	c	2.78	b	2.20	a	0.002	bc	0.004	b
P5	70.92	c	11.08	bc	2.89	ab	2.06	a	0.002	bc	0.003	bc
P6	54.33	d	6.94	d	1.90	d	0.93	c	0.002	c	0.003	c
D1*P1	93.25	b	18.23	bc	2.51	ef	0.97	fg	0.002	c-e	0.006	b
D1*P2	99.25	a	30.98	a	3.07	b	2.40	b	0.003	a	0.007	a
D1*P3	98.25	a	19.10	b	3.43	a	2.23	b	0.003	ab	0.004	d
D1*P4	97.50	a	16.89	cd	3.16	b	3.00	a	0.002	cd	0.005	c
D1*P5	98.25	a	15.72	d	3.40	a	3.00	a	0.002	bc	0.004	d
D1*P6	71.75	e	11.06	fg	2.73	d	1.68	c	0.002	c-e	0.004	de
D2*P1	81.00	d	11.94	ef	2.30	g	0.75	gh	0.002	d-f	0.003	e-g
D2*P2	87.00	c	12.69	ef	2.74	d	1.91	c	0.002	bc	0.005	c
D2*P3	88.00	c	13.47	e	2.89	c	1.21	de	0.002	c-e	0.003	g-i
D2*P4	72.75	e	9.10	gh	2.70	d	1.90	c	0.002	c-e	0.004	d-f
D2*P5	58.50	fg	8.01	h	3.20	b	1.63	cd	0.002	d-g	0.003	f-h
D2*P6	50.50	h	4.91	i	1.80	i	0.70	gh	0.002	de	0.003	hi
D3*P1	43.00	i	5.03	i	1.85	i	0.50	h	0.001	hi	0.002	i
D3*P2	56.00	g	9.52	gh	2.66	d	1.72	c	0.002	c-e	0.004	d-f
D3*P3	60.00	f	7.72	h	2.61	de	1.30	ef	0.002	e-g	0.002	ij
D3*P4	56.50	g	5.61	i	2.46	f	1.71	c	0.002	f-h	0.003	hi
D3*P5	56.00	g	9.52	gh	2.07	h	1.57	cd	0.001	g-i	0.003	hi
D3*P6	40.75	i	4.84	i	1.16	j	0.42	h	0.001	i	0.002	j

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Table 4: Comparison of means using Duncan multiple 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)		leaves number		leaf area (cm2)	
D1	97.22	a	2.41	a	16.63	a	17.53	a	0.05	a	0.08	A	0.08	a	12.34	a	4.59	a
D2	87.22	b	2.16	ab	16.18	a	16.17	ab	0.04	ab	0.07	ab	0.07	b	11.33	ab	3.98	ab
D3	78.89	b	1.88	b	15.29	b	13.96	b	0.03	b	0.06	B	0.06	b	11.08	b	3.31	b
P1	76.67	c	1.76	c	13.71	c	14.92	c	0.02	c	0.06	C	0.06	d	10.62	c	3.34	a
P2	88.89	b	2.18	bc	17.84	a	17.11	a	0.05	a	0.09	A	0.09	a	12.50	a	4.42	a
P3	86.67	bc	2.12	bc	16.51	ab	15.98	bc	0.04	b	0.08	abc	0.07	c	11.92	a-c	4.15	a
P4	88.89	b	1.96	bc	15.48	bc	15.32	c	0.04	ab	0.08	ab	0.08	b	11.00	bc	3.74	a
P5	98.89	a	2.63	a	16.50	ab	16.48	ab	0.05	ab	0.07	bc	0.07	bc	12.07	ab	4.20	a
P6	86.67	bc	2.26	ab	16.16	ab	15.52	bc	0.04	b	0.06	bc	0.06	cd	11.41	a-c	3.90	a
D1*P1	93.33	a-c	2.06	e-i	15.07	fg	16.52	ab	0.03	f-h	0.06	d	0.06	ef	10.87	de	3.91	a-e
D1*P2	100.00	a	2.61	a-c	18.33	a	18.87	a	0.06	a	0.10	a	0.10	a	13.20	a	4.94	ab
D1*P3	96.67	ab	2.51	a-d	16.80	a-f	18.80	a	0.05	ab	0.10	a	0.07	c	13.23	a	5.15	a
D1*P4	96.67	ab	2.14	d-h	16.20	b-f	17.54	ab	0.05	a-d	0.10	a	0.09	b	11.80	b-e	4.29	a-e
D1*P5	100.00	a	2.42	a-e	17.50	a-d	16.34	ab	0.06	a	0.08	abc	0.08	b	12.90	ab	4.95	ab
D1*P6	96.67	ab	2.75	a	15.89	c-f	17.13	ab	0.05	a-d	0.07	cd	0.07	c-f	12.07	a-d	4.31	a-d
D2*P1	73.33	gh	1.70	h-j	13.50	gh	14.93	b-d	0.02	hi	0.05	d	0.05	g	10.50	e	3.21	c-e
D2*P2	86.67	b-e	2.11	d-h	18.10	ab	17.17	ab	0.05	a-c	0.09	ab	0.09	b	12.30	a-c	4.51	a-c
D2*P3	86.67	b-e	2.20	c-g	17.73	a-c	15.67	bc	0.04	e-g	0.07	bcd	0.07	cde	12.03	a-d	4.48	a-c
D2*P4	86.67	b-f	1.89	f-j	15.25	e-g	17.40	ab	0.04	c-g	0.09	ab	0.07	cd	10.40	e	3.75	a-e
D2*P5	100.00	a	2.79	a	15.71	d-f	16.42	ab	0.05	a-e	0.06	cd	0.06	fg	11.60	b-e	3.97	a-e
D2*P6	90.00	a-d	2.27	b-f	16.80	a-f	15.44	b-d	0.04	b-e	0.06	cd	0.06	ef	11.17	c-e	3.95	a-e
D3*P1	63.33	h	1.51	j	12.57	h	13.30	cd	0.01	i	0.06	d	0.05	g	10.50	e	2.91	de
D3*P2	80.00	d-g	1.82	g-j	17.10	a-e	15.30	b-d	0.05	a-c	0.07	bcd	0.09	b	12.00	a-d	3.82	a-e
D3*P3	76.67	eg	1.64	ij	15.00	fg	14.98	b-d	0.03	gh	0.06	d	0.05	g	10.50	e	2.83	e
D3*P4	83.33	c-g	1.86	f-j	15.00	fg	13.00	d	0.04	d-g	0.07	cd	0.07	def	10.80	de	3.17	c-e
D3*P5	96.67	ab	2.68	ab	16.30	b-f	13.79	cd	0.04	b-f	0.06	cd	0.06	ef	11.70	b-e	3.67	b-e
D3*P6	73.33	gh	1.77	g-j	15.80	c-f	13.40	cd	0.03	gh	0.06	cd	0.06	fg	11.00	c-e	3.44	c-e

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Lower endosperm analysis and thus low assimilate transport to embryo results in shorter shoots during osmotic stress conditions (Trautwein *et al.*, 1997).
Root and shoot dry weight affected by drought and priming ($p<0.01$), shoot dry weight also affected by interaction between treatments ($p<0.05$) (table 2). The highest and lowest root dry weight produced by sin-stress and -15 bar osmotic stress treatments respectively (table 4).
Shoot dry weight decreased by drought, but primed seeds produced heavier shoots compared with non-primed seeds. Between different priming treatments the highest root and shoot weight observed for hydro-primed seeds.
The highest shoot dry weight of primed seeds may be due to the faster emergence of primed seeds which results in better water and nutrition absorbance and different plant growth behaviors (Harris *et al.*, 2000). The results were in agreement with Basra *et al.*, (2004) which reported significant effect of seed priming in dry weight compare with control weeds.
Leaves number and leaf area per plant affected by drought ($p<0.01$). Leaves number affect by priming level too ($p<0.01$). Leaves dry weight affect by drought, priming and interaction between them ($p<0.01$) (table 2). Leaves number and area decreased by drought (table 4). Priming results in higher leaves number per plant. The highest leaves number produced by hydro-priming (table 4). Leaf area reduction is a regulatory method of plants faced to drought condition (Levitte, 1980). Plant water content controls by reducing transpiration rate via producing lower leaves number (Hopkins, 1999).
Leaf dry matter decreased by drought and priming diminished suppressing effect of drought on leaf dry matter. The higher leaf dry weight obtained by hydro-priming followed by applying -3 and -6 bar solutions of PEG6000 (figure 4).

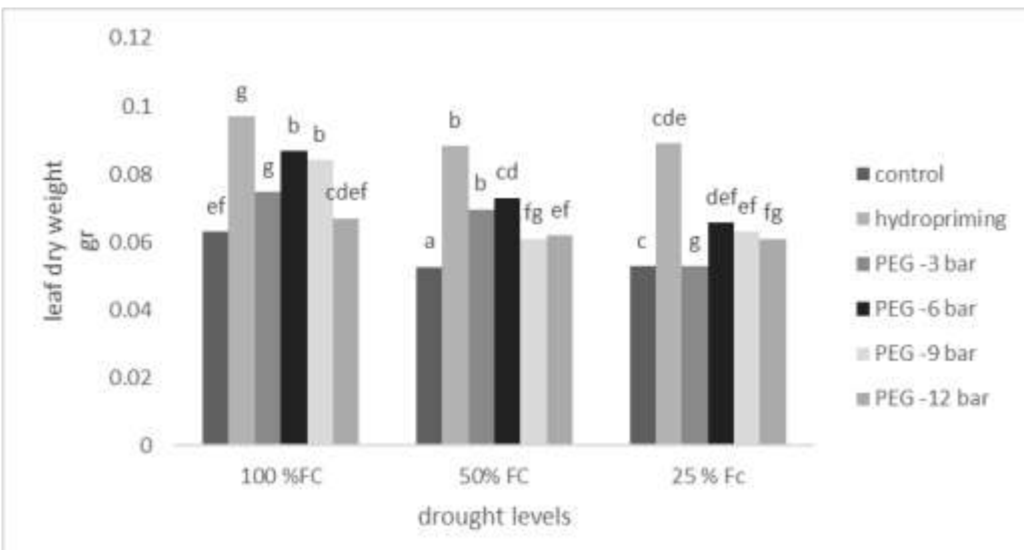

Figure 4: leaf dry weight as affected by drought and priming

CONCLUSION

Results showed that *Nigella sativa* is sensitive to drought during germination stage. Higher drought intensity resulted in lower germination rate and percentage and growth parameters. However, this reduction in germination and growth parameter was significantly higher for nonprime seeds, compared to primed seeds. Drought reduced dry matter accumulation of plant by reducing photosynthetic capacity due to lower leaves number and area. Between priming solutions the best results seen for hydro-priming in respect of germination rate and percentage and dry weight. Higher osmotic potential of priming solutions showed less advantage in respect of shoot and root dry weight and germination rate compare with -3 and -6 bar solutions of PEG6000. Root and shoot length and dry weight enhanced in primed seeds due to extended time of growth which was the result of accelerated germination.

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