

## STUDY OF GERMINATION AND SEEDLING GROWTH OF BLACK CUMIN (*NIGELLA SATIVA* L.) TREATED BY HYDRO AND OSMOPRIMING UNDER SALT STRESS CONDITIONS

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**ABSTRACT.** The objective of the study was to determine the responsible factors for germination and early seedling growth due to salt toxicity or osmotic effect and to optimize the best priming treatment for these stress conditions. To study the effect of osmopriming and hydropriming on germination and seedling growth of black cumin (*Nigella sativa* L.) under salt stress conditions this experiment was conducted at Torbat-Heydariyeh University, Torbat, Iran. The treated seeds (control, hydropriming and ZnSO<sub>4</sub>) of black cumin were evaluated at germination and seedling growth for tolerance to salt (NaCl and Na<sub>2</sub>SO<sub>4</sub>) conditions at the same water potentials of 0.0, -0.3, -0.6, -0.9 and -1.2MPa. Electrical conductivity (EC) values of the NaCl solutions were 0.0, 6.5, 12.7, 18.4 and 23.5 dSm<sup>-1</sup>, respectively. Results showed that hydropriming increased germination and seedling growth under salt stress. Germination delayed in both solutions, having variable germination with different priming treatments. In NaCl treatment, germination percentage, root and shoot

weight, shoot and root length were higher but mean germination time and abnormal germination percentage were lower than Na<sub>2</sub>SO<sub>4</sub>, at the same water potential. The root / shoot weight and R/S length enhanced with increase of osmotic potential in both NaCl and Na<sub>2</sub>SO<sub>4</sub> solutions. NaCl had less inhibitor effect on seedling growth than the germination. It was concluded that inhibition of germination at the same water potential of NaCl and Na<sub>2</sub>SO<sub>4</sub> resulted from salt toxicity rather than osmotic effect. The findings of this experiment can be useful and applied to achieve best germination and uniform emergence under field conditions for farmers of medicinal plants.

**Key words:** Black cumin (*Nigella sativa* L.); Salt stress; ZnSO<sub>4</sub>; Priming; Seedling.

### INTRODUCTION

The black cumin (*Nigella sativa* L.), belonging to the family Ranunculaceae is an ancient crop which is originated in the East-

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southern Europe. It is particularly grown in southern Europe (The Balkans), northern Africa and Hindustan. The black cumin is generally short-lived annual, typical of disturbed soils or natural communities of semiarid regions, with a dominance of therophytes. In the natural form, flowers are bluish, with a variable number of multi-ovule carpels, developing into a follicle after pollination, with single fruits, partially connected to form a capsule structure. Seeds, of generally small size (1-5 mg), dark grey or black color and with corrugated integuments, represent the useful product (Michel, 1983; El-Fouly, 1983).

A major constraint to seed germination is soil salinity, a common problem in irrigated areas of Iran, with low rainfall (Kaya *et al.*, 2003; Kafi, 2002; Kizil *et al.*, 2003; Mehra *et al.*, 2003; Mwale *et al.*, 2003). Soil salinity may affect the germination of seeds either by creating an osmotic potential external to the seed, preventing water uptake, or through the toxic effects of Na<sup>+</sup> and Cl<sup>-</sup> ions on the germinating seed (Khajeh-Hosseini *et al.*, 2003). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri *et al.*, 2001; Angadi *et al.*, 2002). Under these stresses there is a decrease in water uptake during imbibition and, furthermore, salt stress may cause excessive uptake of ions (Murillo-Amador *et al.*, 2002).

Ahmadian *et al.* (2009) reported that hydropriming and osmopriming

with NaCl and Na<sub>2</sub>SO<sub>4</sub> solutions increased germination percentage and rate and primary seedling growth under salt stress. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses (Heydecker and Coolbaer, 1977; Bradford, 1986). The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, soybean and sunflower (Parera and Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini *et al.*, 2003; Sadeghian and Yavari, 2004). Dharmalingam and Basu (1990) reported beneficial effect of a hydration-dehydration seed treatment on germination of sunflower. Rao *et al.* (1987) reports that primed *Brassica* seeds may reduce the risk of poor stand establishment in cold and moist soils. However, Singh and Rao (1993) stress that KNO<sub>3</sub> effectively improved germination, seedling growth and seedling vigour index of the seeds of sunflower varieties with low germination.

The aims of the present study were to determine the responsible factors for failure of germination of black cumin seeds under saline conditions due to the toxic effects of NaCl and Na<sub>2</sub>SO<sub>4</sub> by comparing seed germination under a range of osmotic potentials due to NaCl and Na<sub>2</sub>SO<sub>4</sub>. Furthermore, the study examined the possibilities to overcome salt stress by seed treatments with hydropriming or treatment with ZnSO<sub>4</sub>.

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### MATERIALS AND METHODS

This experiment was carried out at the Department of Medicinal plants, Faculty of Agriculture, University of Torbat-e Heydariyeh, Iran in 2012. Germination and early seedling growth (7 days) of the cultivar were studied using distilled water (control) and under osmotic potentials of -0.3, -0.6, -0.9 and -1.2MPa, for NaCl (Coons *et al.*, 1990) or polyethylene glycol (PEG 6000) (Michel and Kaufmann, 1973). NaCl concentrations had the electrical conductivity (EC) values of 6.5, 12.7, 18.4 and 23.5 dSm<sup>-1</sup>, respectively.

#### Seed treatments

For hydropriming, cumin seeds (4.4% seed moisture) were immersed in distilled water at 25°C, for 18 h, under dark conditions. The hydropriming duration was determined by controlling seed imbibition during germination. For ZnSO<sub>4</sub> treatment, the seeds were immersed in 500 ppm ZnSO<sub>4</sub> solution at 25°C, for 2 h, in the dark (Singh and Rao, 1993). Thereafter, the seeds were rinsed with tap water three times. The treated seeds were surface-dried and dried back to their original moisture content at room temperature (about 22°C, 45% relative humidity) determined by changes in seed weight. Moisture content of untreated seeds (control, 4.4% moisture content), hydroprimed and ZnSO<sub>4</sub> treated seeds was equilibrated at room temperature for 2 days.

#### Germination tests

Three replicates of 25 seeds were germinated between double layered rolled Anchor germination papers with 10 ml of respective test solutions. The papers were replaced every 2 days to prevent accumulation of salts (Rehman *et al.*,

1996). The rolled paper with seeds was put into sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate at 25±1°C, in the dark, for 7 days. Germination was considered to have occurred when the radicles were 2 mm long. Germination percentage was recorded every 24 h, for 7 days. To determine the toxic effects of the solutions on germination, non-germinated seeds in each treatment were transferred to distilled water and counted 3 days later. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980). The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated (ISTA, 2003).

Root length, shoot length and seedling fresh weights were measured after the 7th day. Three grams of the seeds from each seed treatment were placed in Petri dishes, containing distilled water to determine water uptake of seeds necessary for germination. The water uptake was expressed as the percentage increase in moisture content on fresh weight basis.

#### Experimental design

The experimental design was three factors factorial (3×2×5), arranged in a complete randomized design with three replications and 25 seeds per replication. The first factor was seed treatment (control, hydropriming and ZnSO<sub>4</sub>), the second, solutions of NaCl and Na<sub>2</sub>SO<sub>4</sub> and the third was osmotic potential level (0, -0.3, -0.6, -0.9 and -1.2 MPa). Data for germination percentage were subjected to arcsine transformation, and analysis of variance was made using MSTAT-C program (Michigan State University). The differences between the means were compared using LSD values ( $P < 0.05$ ).

## RESULTS

A significant three-way interaction (seed treatment, solution and stress) was found ( $P < 0.05$ , 60 d.f.) for all investigated characters. The germination rate decreased with decrease in osmotic potential in both NaCl and Na<sub>2</sub>SO<sub>4</sub> solution; however, Na<sub>2</sub>SO<sub>4</sub> delayed it more compared to NaCl (Table 1). Both priming treatments increased the rate of seed germination. Hydropriming resulted in the accelerated germination for both NaCl and Na<sub>2</sub>SO<sub>4</sub>, especially under low osmotic potential. Water uptake of primed seeds did not change significantly ( $P < 0.05$ ) (data not shown), while the time to seed germination for hydropriming, ZnSO<sub>4</sub> and control was delayed by 12, 18 and 38 h, respectively (data not shown).

Germination percentage was influenced by salt stresses, but inhibition was greater in Na<sub>2</sub>SO<sub>4</sub> (Table 2). Hydropriming showed maximum germination under all osmotic potential of NaCl solutions. Germination percentage drastically declined and delayed with increase of osmotic stress due to Na<sub>2</sub>SO<sub>4</sub> in MPa lower than -0.6 and NaCl in MPa lower than -0.9. Considering seed treatments, hydropriming gave higher germination percentage in Na<sub>2</sub>SO<sub>4</sub> solution. Transfer of non-germinated seeds from PEG solution to the distilled water resulted in 100% germination regardless of osmotic potential (data not shown). ZnSO<sub>4</sub> and hydropriming diminished the abnormal germination in NaCl and Na<sub>2</sub>SO<sub>4</sub>. Increased water stress was accompanied with increase of abnormal germination in both solutions potential (data not shown).

**Table 1 - Germination rate (seed/days) of black cumin seeds treated with ZnSO<sub>4</sub>, hydropriming and control (untreated) at salt stress of NaCl and Na<sub>2</sub>SO<sub>4</sub>**

MPa	Seed treatments					
	Control (untreated)		Hydropriming		ZnSO <sub>4</sub>	
	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>
0	7.76	7.76	10.13	10.13	7	7
-0.3	6.53	5.8	9.36	7.33	6.15	5.1
-0.6	5.4	3.73	8.3	6.3	4.73	3.33
-0.9	3.76	2.5	7.2	4.63	4	2.17
-1.2	2.33	1.37	6	3.13	2.47	1.29

Although root length was affected due to salt stress, significant and higher inhibition due to Na<sub>2</sub>SO<sub>4</sub> was very evident ( $P < 0.05$ ; Table 3). At -1.2 MPa, radicle growth stopped after emergence of radicle or primary

root from the seed. Greater reduction in shoot length due to Na<sub>2</sub>SO<sub>4</sub>, compared to NaCl, was very evident ( $P < 0.05$ ), with no recorded shoot growth at -0.9 MPa of Na<sub>2</sub>SO<sub>4</sub> (Table 4). However, hydropriming enhanced

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shoot growth at -0.9 MPa of Na<sub>2</sub>SO<sub>4</sub>. Also, hydropriming exhibited higher shoot growth due to all concentrations of NaCl. Depending on decrease in shoot and root length, shoot and root weight gradually declined with the decreasing osmotic potential of solutions (Tabs. 5 and 6). Higher

shoot and root weights were recorded from NaCl, compared to osmotic stress at -0.6 MPa due to Na<sub>2</sub>SO<sub>4</sub> and above. The root / shoot weight and R/S length increased with increase in osmotic potential in both NaCl and Na<sub>2</sub>SO<sub>4</sub> solution (Fig. 1).

**Table 2 - Germination percentage of seeds treated with ZnSO<sub>4</sub>, hydropriming and control (untreated) at salt stress of NaCl and Na<sub>2</sub>SO<sub>4</sub>**

MPa	Seed treatments					
	control (untreated)		Hydropriming		ZnSO <sub>4</sub>	
	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>
0	56	56	69	69	59	59
-0.3	51	46	66	66	58	57
-0.6	37	29	52	49	47	45
-0.9	28	9	44	19	36	16
-1.2	10	6	16	13	13	12

**Table 3 - Root length (cm) of black cumin seedlings treated with ZnSO<sub>4</sub>, hydropriming and control (untreated) at salt stress of NaCl and Na<sub>2</sub>SO<sub>4</sub>**

MPa	Seed treatments					
	Control (untreated)		Hydropriming		ZnSO <sub>4</sub>	
	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>
0	1.87	1.87	1.83	1.83	1.80	1.80
-0.3	1.45	1.42	1.78	1.58	1.82	1.74
-0.6	1.22	1.12	1.52	1.28	1.68	1.41
-0.9	0.84	0.52	1.00	0.72	1.44	0.67
-1.2	0.54	0.00	0.66	0.00	0.69	0.00

**Table 4 - Shoot length (cm) of black cumin seedlings treated with ZnSO<sub>4</sub>, hydropriming and control (untreated) at salt stress of NaCl and Na<sub>2</sub>SO<sub>4</sub>**

MPa	Seed treatments					
	Control (untreated)		Hydropriming		ZnSO <sub>4</sub>	
	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>
0	1.54	1.54	1.38	1.38	1.46	1.46
-0.3	1.36	0.96	1.13	1.05	1.18	1.12
-0.6	0.87	0.68	0.79	0.68	0.86	0.67
-0.9	0.59	0.32	0.38	0.29	0.46	0.21
-1.2	0.20	0.00	0.23	0.00	0.23	0.00

**Table 5 - Root weight (mg plant<sup>-1</sup>) of black cumin seedlings treated with ZnSO<sub>4</sub>, hydropriming and control (untreated) at salt stress of NaCl and Na<sub>2</sub>SO<sub>4</sub>**

MPa	Seed treatments					
	Control (untreated)		Hydropriming		ZnSO <sub>4</sub>	
	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>
0	0.69	0.69	0.89	0.89	0.77	0.77
-0.3	0.56	0.45	0.82	0.78	0.67	0.65
-0.6	0.55	0.32	0.66	0.62	0.55	0.52
-0.9	0.22	0.2	0.53	0.47	0.32	0.33
-1.2	0.13	0.00	0.18	0.00	0.16	0.00

**Table 6 - Shoot weight (mg plant<sup>-1</sup>) of cumin seedlings treated with ZnSO<sub>4</sub>, hydropriming and control (untreated) at salt stress of NaCl and Na<sub>2</sub>SO<sub>4</sub>**

MPa	Seed treatments					
	Control (untreated)		Hydropriming		ZnSO <sub>4</sub>	
	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>
0	0.44	0.44	0.64	0.64	0.52	0.52
-0.3	0.31	0.2	0.57	0.53	0.42	0.4
-0.6	0.3	0.17	0.41	0.37	0.3	0.27
-0.9	0.16	0.11	0.28	0.22	0.07	0.08
-1.2	0.12	0.00	0.17	0.00	0.06	0.00

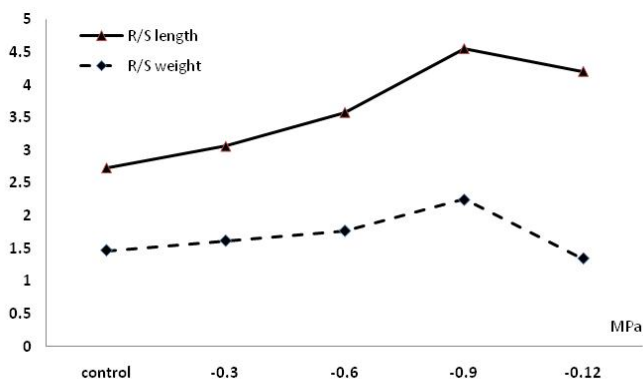


Fig 1. Root/Shoot weight and length of black cumin treated with ZnSO<sub>4</sub>, hydropriming and control (untreated) at salt stress of NaCl and Na<sub>2</sub>SO<sub>4</sub>.

## DISCUSSION

Both seed treatments showed enhanced performance under stress conditions. Germination rate was

increased by seed priming, but stress conditions delayed it considerably. Compared to Na<sub>2</sub>SO<sub>4</sub>, germination rate for NaCl was more at equivalent osmotic potential. This could be

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explained by more rapid water uptake in hydroprimed seeds because germination for hydropriming, ZnSO<sub>4</sub> and control started at 12, 18 and 38 h, respectively (data not shown). It supports that hydropriming caused more rapid water uptake than the amount of water for germination. The results are in line with the findings of Ahmadian *et al.* (2009) in cumin. Sung and Chiu (1995) observed that mean germination time was accelerated by hydropriming without changing amount of water uptake in watermelon.

Hydropriming clearly improved both rate of germination and mean germination time both under salt stress conditions. Furthermore, hydropriming resulted in increase of normal germination. The results are in line with the findings of Thornton and Powell (1992) in *Brassica*, Srinivasan *et al.* (1999) in mustard, and Ahmadian *et al.* (2009) in cumin. Fujikura *et al.* (1993) indicated the beneficial effects of hydropriming on aged seeds, with respect to germination and percentage of normal seedlings, in cauliflower. Furthermore, Sadeghian and Yavari (2004) reported that increasing drought stress resulted in increasing abnormal seedlings in sugar beet. It is concluded that superiority of hydropriming on germination could be due to soaking time effects rather than ZnSO<sub>4</sub> treatment. Because hydroprimed seeds compared to ZnSO<sub>4</sub> treated seeds were allowed to imbibe water for a longer time and went through the first stage of

germination without protrusion of radicle. Akinola *et al.* (2000) reported that higher duration of exposure to seed treatment resulted in higher cumulative germination in wild sunflower and Caseiro *et al.* (2004) found that hydropriming was the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 h, compared to 48 h. The beneficial effects of hydropriming on germination were found in this study. ZnSO<sub>4</sub> shortened MGT, however, final germination was higher from hydropriming, suggesting toxicity of ZnSO<sub>4</sub> due to ion accumulation in the embryo (Demir and Van De Venter, 1999).

Seeds always germinated better in NaCl than Na<sub>2</sub>SO<sub>4</sub> at the equivalent water potential in line with earlier observations made for soybean by Khajeh-Hosseini *et al.* (2003), and for cumin by Ahmadian *et al.* (2009). This may be due to the uptake of Na<sup>+</sup> and Cl<sup>-</sup> ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination. Lower germination percentage obtained from Na<sub>2</sub>SO<sub>4</sub> compared with NaCl, at equivalent water potential in each priming method suggest that adverse effects of Na<sub>2</sub>SO<sub>4</sub> on germination were due to specific ion accumulation rather than osmotic effect. These results agree with Murillo-Amador *et al.* (2002) in cowpea, Demir and Van De Venter (1999) in watermelon, they affirmed that drought or salinity may influence germination by decreasing the water

uptake and toxicity of ions. Under salt stress,  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{SO}_4^-$  may be taken up by the seed and toxic effect of  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  might appear. However, our findings at high salinity concentration of  $23.5 \text{ dSm}^{-1}$  showed that decrease in germination percentage was significant.

The main effect of seed treatments was an increase in germination rate; however, post-germination growth was also increased. Hydropriming improved seedling fresh weight, under osmotic stress. Considering both seed treatments, it was concluded that hydropriming improved root growth and gave the highest root length in both solutions. El-Midaoui *et al.* (2003) reports that root and shoot growth significantly decreased by osmotic stress, at  $-0.6\text{MPa}$ , and above induced by PEG 6000. Murillo-Amador *et al.* (2002) found that seedling growth of cowpea was inhibited by both  $\text{NaCl}$  and PEG, but higher inhibition occurred due to PEG. Sung and Chiu (1995) proposed that emergence force and seedling growth were strengthened by hydropriming in watermelon. Seedling growth severely diminished with increased drought stress and genetic differences were found in sugar beet (Sadeghian and Yavari, 2004).

In many coated seeds, germination and subsequent seedling growth can be inhibited by mechanical restriction exerted by the seed coat (Sung and Chiu, 1995). Priming may be helpful in reducing

the risk of poor stand establishment under drought and salt stress and permit more uniform growth under conditions of irregular rainfall and drought on saline soils.

Parera and Cantliffe (1994) and McDonald (1999) emphasize that hydropriming is the simplest approach to hydrating seeds and minimizes the use of chemicals. However, if the seeds are not accurately hydrated, the rate of hydration cannot be exactly controlled. It was observed that hydropriming practically ensured rapid and uniform germination accompanied with low abnormal seedling percentage in line with Singh (1995) and Shivankar *et al.* (2003). They stress that it has high potential in improving field emergence and ensures early flowering and harvest under stress conditions especially in dry areas.

## CONCLUSIONS

Our findings revealed that inhibition of germination at equivalent water potential of  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  resulted from salt toxicity rather than osmotic effect. Both seed treatments gave better performance than control (untreated) under salt stress with clear effectiveness of hydropriming in improving the germination percentage at low water potential. In commercial production of black cumin in Iran, germination of cumin seeds is a major problem to planting. To achieve a uniform plant density in case of drought, growers tend to sow  $25 \text{ kg ha}^{-1}$  of seed while only  $4.5 \text{ kg ha}^{-1}$  is



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needed. Hydrated seeds with higher germination percentage under salt stress or micronutrient application increased tolerance of seeds to salt stress. In addition, reported protocol is simple, cheap and does not require expensive chemicals and sophisticated equipment. The protocol has practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions.

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