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Effect of Salinity Stress on the Germination, Growth and Some Physiological Activities of Black Cumin (*Nigella sativa* L.)

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ABSTRACT. The effect of different salinity levels (up to 300 mM NaCl) on germination, growth and some metabolic parameters of black cumin (*Nigella sativa* L.) was studied. The plant exhibited a good tolerance to salinity up to 150 mM during germination. But however, fresh and dry weight of shoot and root, Photosynthetic Pigments and leaf area were decreased when treatments were greater than 150 mM. Soluble and insoluble carbohydrates and proline contents increased with the increasing NaCl concentrations, whereas amino acids - free proline were reduced. Soluble protein was unaffected under all salinity levels, except in case of roots where it was decreased. Insoluble protein was decreased by increasing salinity. These results may lead to the suggestion that, black cumin is a salt tolerant plant and may be considered a glycophyte.

Salinity is a major problem in today's irrigation agriculture, as millions of tons of salts are annually deposited onto the soil from irrigation water. Adverse effects of salt stress on seed germination, seedling growth, as well as some related physiological activities of a number of cultivated plant species have been subjected to extensive investigations (Greenway and Munns 1980, Epstein 1983, Shah *et al.* 1987, Hampson and Simpson 1990, Zidan 1991, Schmidhalfer and Oertti 1991). However, the trends and magnitudes of these effects vary according to the level and duration of salinization treatment as well as the plant type used *e.g.* (Heikal *et al.*

1992, Dell'Aquila and Spada 1993 and Hajar *et al.* 1993). This variation in plant response and the need to select some of our economic plants for cultivation in saline soils, necessitated a series of investigations to test their salinity tolerance and to elaborate on their physiological adaptation to salinity.

Plants apparently rely on several mechanisms by which they adapt to salinity stress. These include accumulation of organic molecules like soluble carbohydrates, soluble proteins, proline and possibly other compounds which may act as non-toxic cytoplasmic osmotica in various salt-tolerant plants (Gibson 1988, Shaddad and Zidan 1989, Shah *et al.* 1990 and Zidan 1991).

Black cumin *(Nigella sativa)* is regarded as an important medicinal plant, highly valued for its antimicrobial constituent called nigellone (Chakravarty 1993). In addition, Al-Jassir (1992) reported on the high nutritional potential of Saudi black cumin seeds especially as a source of protein and fat. Nothing is known about the effects of salinity on this important crop.

The objective of the present study is therefore to evaluate the effects of different levels of salinity on seed germination, plant growth (dry matter and photosynthetic pigments) and some metabolic changes in shoots and roots of black cumin.

Materials and Methods

Sodium chloride is known to be the most common salt associated with saline soils. It's salinity effect on seed germination, growth and some physiological activities of black cumin *Nigella sativa* have been tested in the present work. Germination of seeds was carried out according to Mafton and Sepaskhah (1978). The following salinity levels, 0.0, 50, 100, 150, 200, 250 and 300 mM NaCl were used. Under 25 ± 2°C temperature, seeds were placed on absorbant pads in petri dishes containing 30 ml of the appropriate solution 4 replicates of 25 seeds were used in each treatment. Seed was considered to be germinated by the emergence of the radicle.

Seeds of black cumin were grown in peat moss soil, under field capacity watering regime until emergence of the primary leaves, at which time equal number of seedlings were transplanted into 20 cm. diameter pots, containing support media (peat moss soil). Pots were placed in trays containing half strength nutrient, (Hogland and Arnon 1950). The plants were grown on this control solution for 10 days, at which time salinization was started. The pots of the tested plants were divided into seven groups, of four pots and then watered with half strength Hogland solution containing various concentrations of NaCl (0.0, 50, 100, 150, 200, 250 and 300 mM). Every two days, each group was irrigated with the respective saline solution. All pots were leached weekly with excessive amount of tap water. The work carried out in a controlled environment growth chamber maintained at 25 ± 2°C during day and night with relative humidity 60-70%, and with a 14 hour photoperiod of 600-700 μ mol m^{-2} s^{-1} at plant height. All plants used in experiments was maintained at their final salinity for 25 days before harvest.

At the end of the experiment photosynethetic pigments were determined according to Mitzner *et al.* (1965). Leaf area was measured using a portable area meter (Li-3000). The plants were harvested and weighed fresh. Each plant was then separated into leaves, stems and roots and oven-dried at 75°C constant weight. The dry weight were recorded, the shoot/root ratios derived.

Free proline was determined following the method descried by Bates *et al.* (1973). Carbohydrat determination was according to Fales (1951). The insoluble and soluble protein were measured according to Lowry *et al.* (1951). Mitchell *et al.* (1976) method was used in the determination of amino acids (Free proline). Four replicates were used in each reading and the data were then statistically analysed, and the least significant difference (L.S.D.) were calculated.

Results and Discussion

Seed germination

The most striking feature of this investigation is the tolerance of black cumin seeds to moderate levels of salinity. The final germination percentage was found to be unaffected up to 150 mM NaCl. The data in Table (1) reveal that the final germination percentage of black cumin remained unchanged up to the level of 150 mM NaCl, thereafter the values tended to decrease with the increase of NaCl level. At lower salinity levels (50 to 150 mM NaCl), most seeds germinated within one to four days but germination was progressively delayed as the salinity increased, and a final germination percentage of 60 and 50% required up to 6 days at higher salinity levels (250 and 300 mM NaCl respectively). Similar findings of reduced seed germination due to water stress induced by increasing salt concentrations have been reported by (Heikal *et al.* 1981, Dell'Aquila 1992 and Malibari *et al.* 1993).

Growth:

The values of the growth parameters (fresh weight, dry weight, leaf area) of black cumin were found to be generally lowered with the rise of salinization level (Table 2). However, low levels (50 and 100 mM NaCl) of salinization stimulated

Table 1. Effect of various concentrations of NaCl on the germination of black cumin seeds. (Data expressed as %)

NaCl mM	Days					
	1	2	3	4	5	6
0	48	75	100	–	–	–
50	51	81	100	–	–	–
100	43	70	92	100	–	–
150	40	65	80	100	–	–
200	0	15	31	45	62	80
250	0	0	20	37	53	60
300	0	0	10	30	45	52

Table 2. Effect of various concentrations of NaCl on fresh and dry weight (gm/plant) and leaf area (cm^2/plant) of black cumin

NaCl mM	Leaf area	Fresh weight		Dry weight		Shoot/ Root
		Shoots	Roots	Shoots	Roots	
0	3.40	6.23	2.43	1.21	0.48	2.52
50	3.51	6.81	2.95	1.30	0.52	2.50
100	3.51	6.75	2.81	1.36	0.58*	2.35
150	3.11	6.52	2.53	1.11	0.44	2.52
200	2.55*	5.12*	2.05	0.88*	0.31*	2.84*
250	2.15*	4.73*	1.87	0.63*	0.21	3.00*
300	2.00*	3.22*	1.28	0.52*	0.16*	3.25*
L.S.D at 5%	0.31	0.71	0.65	0.14	0.09	0.45

* Significant differences compared with the control.

these parameters. This inhibitory effect of salinity on the growth of black cumin add more support to the results obtained by some other authors using various plants (Epstein 1983, Zidan 1991, Malibari 1993). In addition it was observed that roots of black cumin were affected by salinity more severely than its shoots at treatments greater than 150 mM NaCl. This can be seen from the increased values of shoot/root ratio of salinized plants which were also reported by (Ahmed *et al.* 1980, Zidan 1991).

The contents of the photosynthetic active pigments (chlorophyll a, chlorophyll b and carotenoids) of leaves of test plant harvested 25 days after treatment are given in Table (3). It can be seen that generally there were slight differences in the pigment fractions and consequently in the total pigment content of the plants subjected to the various levels of salinity. However, the contents of different pigment fraction of plants subjected to 250 and 300 mM NaCl were considerably lowered. On the other side, the rate of biosynthesis of chlorophyll a and chlorophyll b could be tested using the values of chl.a/chl.b ratios. It can be seen that in general salinized black cumin plants exhibited lower values of a/b ratio than that of the control plants. Virgin (1965) attributed the reduction in chlorophyll a content under salt stress to inhibition of protochlorophyll synthesis.

Table 3. Effect of various concentrations of NaCl on photosynthetic pigments (mg/gm dry weight) of black cumin

NaCl mM	Chlorophyll a	Chlorophyll b	Carotenoid	Total pigments	Chl.a / Chl.b
0	8.32	4.06	2.83	15.21	2.05
50	8.87	4.13	2.65	15.65	2.15
100	7.92	3.96	2.99	14.87	2.00
150	8.31	4.08	2.80	15.19	2.08
200	7.50	3.96	2.60	14.06	1.89
250	6.21	3.76	2.11	12.08	1.65
300	6.28	3.69	2.15	12.12	1.70
L.S.D at 5%	1.75	1.05	0.97	3.85	0.43

The contents of soluble and insoluble carbohydrates of the variously treated black cumin plants are given in Table (4). It can be seen that the contents of carbohydrates (soluble and insoluble) in the different organs tended to be increased with the increase of salinity level. Many plants which are stressed by salinity accumulate starch and soluble carbohydrates (Greenway and Munns 1980, Rathert 1984). This accumulation has been attributed to an impaired carbohydrate utilization (Munns and Termaat 1986).

Table 4. Effect of various concentrations of NaCl on soluble and insoluble carbohydrates (mg/gm dry weight) of black cumin plants

NaCl mM	Leaves		Stems		Roots	
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
0	96.21	122.3	72.21	85.17	45.22	89.11
50	96.93	120.9	75.11	86.29	43.21	91.45
100	105.05	123.9	81.89	85.35	47.28	89.57
150	112.01	125.9	76.43	87.30	53.21	93.63
200	110.51	125.3	83.52	88.05	52.90	95.54
250	117.62*	124.2	78.71	87.19	55.29	91.21
300	109.73	125.6	81.32	89.29	51.33	91.08
L.S.D at 5%	7.31	10.22	5.54	5.68	4.36	6.01

* Significant differences compared with the control.

The content of insoluble protein of black cumin plant leaves and stems were appreciably decreased by salinity stress, while the soluble protein fraction did not show considerable change, under all salinity stress levels (Table 5). In the case of roots, the soluble protein was decreased, while insoluble protein was increased with the increase of salinity level.

There is a general increaseing trend in the concentration of proline in the different organs of black cumin plants with increasing salinity levels (Table 6). This increase was more prominent at the higher salinity levels. The pattern of changes in the total amino acids . Free proline of the seedlings was opposite to that obtained for changes in proline (Table 6).

The increase in proline under saline conditions observed in these experiments could be at the expense of reduced nitrogen compounds. This conclusion is in accordance with the results obtained by Singh *et al.* (1972) and Shah *et al.* (1990) they pointed out that the accumulation of proline is a metabolic adaptation, which confers survival value, by relieving stress.

In the present investigation, the tolerance of this studied plant during growth might be linked to the accumulation of carbohydrates and proline, which in turn increased their ability for water absorption under salinity stress.

Table 5. Effect of various concentrations of NaCl on soluble and insoluble proteins (mg/gm dry weight) of black cumin

NaCl mM	Leaves		Stems		Roots	
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
0	15.31	19.12	10.11	16.01	7.25	12.25
50	16.21	18.34	11.31	15.19	7.09	12.61
100	15.99	16.54	10.66	15.39	6.31	13.19
150	16.22	15.72	12.44	13.22	6.73	15.08
200	15.33	16.85	9.89	14.38	6.26	14.52
250	17.11	12.91*	10.37	12.71	4.71*	16.47*
300	16.24	14.28	11.02	12.89	5.23	15.22
L.S.D at 5%	1.93	2.51	1.55	2.05	1.09	1.43

* Significant differences compared with the control.

Table 6. Effect of various concentrations of NaCl on proline and other amino acids (mg/gm dry weight) of black cumin

NaCl mM	Leaves		Stems		Roots	
	Amino acids	Proline	Amino acids	Proline	Amino acids	Proline
0	1.12	0.78	0.95	0.58	1.00	0.62
50	1.05	0.78	0.95	0.60	1.00	0.67
100	1.0	0.80	0.90	0.59	0.93	0.65
150	0.88	0.90	0.84	0.65	0.81*	0.68
200	0.75*	0.88	0.80	0.70*	0.74*	0.73
250	0.75*	0.95*	0.71*	0.73*	0.70*	0.77*
300	0.71*	0.95*	0.70*	0.73*	0.65*	0.85*
L.S.D at 5%	0.16	0.14	0.55	0.11	0.17	0.12

* Significant differences compared with the control.

Drought is well known associate with salinity in countries with arid climate. Further investigations on the combined effect of these two ecological factors on *Nigella sativa* L. are therefore suggested.

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تأثير الضغط الملحي على الإنبات والنمو وبعض الأنشطة الفسيولوجية للحبة السوداء *Nigella sativa* L. (black cumin)

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في هذا البحث تمت دراسة تأثير تراكيز مختلفة من ملح الطعام (NaCl) (من صفر إلى ٣٠٠ مليمول) على الإنبات والنمو وبعض دالات الأيض في نبات الحبة السوداء *Nigella sativa* L. (black cumin) . وقد أبدى هذا النبات مقاومة جيدة للملوحة وحتى تركيز ١٥٠ مليمول وذلك خلال الإنبات . ومع ذلك فإن الانبات ، الوزن الطازج والجاف للمجموع الخضري والجذري ، أصباغ البناء الضوئي ومساحة الورقة قد انخفضت عند ارتفاع تركيز المعاملة الملحية عن ١٥٠ مليمول . أما المحتوى الكلي من الكربوهيدرات والبرولين فقد ازداد بزيادة الملوحة . في حين أن المحتوى من الأحماض الأمينية الحرة قد تناقص بزيادة التركيز الملحي . ولم يتأثر البروتين الذائب بالملوحة عند أي تركيز للمجموع الخضري ولكنه تناقص بزيادة الملوحة في المجموع الجذري في حين تناقص المحتوى من البروتينات غير الذائبة بزيادة الملوحة . على العموم فإن هذه النتائج قد تشجع على الاقتراح أن نبات الحبة السوداء *N. sativa* نبات مقاوم للملوحة مع أنه من النباتات الغير ملحية .