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Effect of Different Levels of Salinity on Growth and Ion Contents of Black Seeds (*Nigella sativa* L.)

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Abstract: A pot experiment was conducted to assess the effect of different salinity levels on growth and ion contents of black seeds (*Nigella sativa* L.) at Shakarganj Botanic Garden, Jhang, Pakistan. There were three levels of NaCl salinity as 0 (control), 3 dS/m and 6 dS/m. All growth attributes such as fresh and dry shoot weights, shoot length decreased with increase in salinity levels, but in contrast root length increased with increase in salinity levels. Na^+, Cl^-, and K^+ concentrations increased with increase in salinity but Ca^{2+} concentration was lower as salinity levels increased. It was concluded that with increase in salinity levels there was a significant reduction in biomass production in black seeds. Pattern of accumulation of ions varied significantly.

Key words: Salinity, growth, ions and black seeds

INTRODUCTION

Salinity is a worldwide problem. This problem is particularly serious in arid and semi-arid regions of the world where most of the developing countries happen to fall (Khan *et al*., 1999). Salinity causes not only differences between the mean yield and the potential yield, but also causes yield reduction from year to year. It affects the plant growth directly through its interaction with metabolic rates and pathways within the plants. It affects plant growth at all stages of development and sensitivity to salinity varies from one growth stage to another. Adverse effects of salt stress on germination, seedling growth as well as some physiological activities of a number of cultivated plant species have been extensively investigated (Hussain, *et al*., 2008; Shah, *et al*., 1987; Ashraf, *et al*., 1994)

Black seeds (*Nigella sativa* L.) is native to Western Asia, it is grown throughout much of Asia and the Mediterranean region for its seeds and as a garden plant (Chevallier, 1996). It is extensively used as spice and medicine. The herb has been regarded as the valuable remedy in hepatic and digestive disorder as well as stimulant. In their external use seeds give relief when bruised in vinegar and applied on pityriasis, leucoderma, ringworm, eczema, alopecia and pimples (Ghani, *et al*., 1997).

Salinity stress disturbs the uptake and accumulation of essential nutrients (Greenway and Munns, 1980; Shannon and Grieve, 1999; Zhu, 2001). Generally, Ca2+ and K+ are decreased in plants under saline conditions (Khan, 1993; Al-Harbi, 1995) In contrast, Ashraf and Rauf (2001) reported that under saline conditions concentrations of Na^+, K^+ and Ca^{2+} increased significantly in all the parts of germinating from maize seeds primed with NaCl, KCl, and $CaCl_2{\cdot}2H_2O$, respectively. Alam and Naqvi (1991) observed that plant height and dry matter yield decreased with increase in salinity at 85-days old plants of black seed under salinity levels of 1.95, 4.69, 9.38 and 14.06 dS m^{-1} NaCl. Salinity caused an increase in N, P, Ca^{++}, Na^+, Fe^{++} and Mn^{++} and decrease in K^+ contents of the leaves.

It is generally accepted that the germination and seedling stage of plant life cycle is more sensitive to salinity than the adult stage (Ashraf, *et al*., 1986). Effect of salinity at different growth stages in wheat, sorghum and cowpea was investigated and it was found that the early seedling period was the most sensitive one in all the crops and reduction in growth was observed which decreased with increase in salinity (Shalhevet, 1995).

In view of these studies, the principal objective to carryout the present study was to assess the effect of salinity on growth and ion contents in black seeds (Fig. 1).

MATERIALS AND METHODS

This study was conducted during 2007-08 at Shakarganj Sugar Research Institute, Jhang, Pakistan and seeds of black seed *(Nigella sativa* L.*)* were obtained from the Qarshi herb Garden, Hattar, Haripur Pakistan. Seeds were surface sterilized by dipping in 10 percent sodium hypochlorite solution for 10 minutes, then rinsed with sterilized distilled water and air-dried at an ambient temperature of 32ºC in the laboratory. Two levels of NaCl salt (0 and 100 mol m^{-3} NaCl) were applied after 14-days of germination. The experiment was laid out as Completely Randomized Block Design (CRBD) with six replicates. Plants were harvested at seedlings stage (21-days after treatment) and vegetative stage (42-days after treatment).

Plants were uprooted carefully and washed in distilled water. Shoot, root and 1st internode length was measured with the help of scale meter in cm at final harvest (42-days after treatment). Plant samples were

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placed in oven at 75ºC. After 4-days shoot and root dry weight (g/pot) was calculated with the help of electric balance at final harvest (42-days after treatment).

Dried plant material was finely ground and digested with a nitric-perchloric mixture. In stems *plus* sheaths, roots, young leaves (2nd leaf starting from the top) and old leaves (3^{rd} leaf starting from the top) ion contents of Na^+ and K+ were determined by emission spectrophotometry and of Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry (Allan, 1969). Data obtained was used to estimate the net ion transfer rates to the shoot according to the equation proposed by Salim and Pitman, (1983) at both harvests (21 and 42 days after treatments).

Chloride was extracted by stirring ground-dried samples with 0.1 M $NaNO_3$ for 30 min. After extract clarification with activated coal, it was added 13.2 mM $Hg(SCN)_2$ in methanol and 20.2% (w/v) $Fe(NO_3)_3$ (4 + 1) and the absorbance determined at 460 nm (Gaines *et al.*, 1984).

Analysis of variance (ANOVA) technique was employed for carrying out statistical analysis of data collected (Steel and Torie, 1980). The means values were compared with Least Significant Difference (LSD) Test, following to Snedecor and Cochran (1980).

The chemical analysis of soil was carried out according to the method described by USDA Salinity Laboratory Staff (1954). The experiment was laid out in a Completely Randomized Block Design (CRBD) with four replications. The salinity levels 0 (control), 3 dS/m and 6 dS/m were applied by using NaCl at the seedling stage (21 days after germination). Salinity levels were developed as

1 mole of NaCl = 58.5 g NaCl dissolved in 1 liter water
1 mol m^{-3} = 0.0585 g NaCl dissolved in 1 liter water
10 mol m^{-3} (1 dS/m) = 0.00585 g NaCl dissolved in 1 liter water
For 3 dS/m
30 mol m^{-3} (3 dS/m) = 0.00583 X 30 g NaCl per pot
For 6 dS/m
60 mol m^{-3} (6 dS/m) = 0.00583 X 60 g NaCl per pot

Plants were harvested at the flowering stage. The data for shoot fresh weight (g), shoot dry weight (g), shoot length (cm) and root length (cm) was recorded.

Ion concentrations: The dried ground plant material (0.1 g) was digested with sulphuric acid (H_2SO_4) and hydrogen peroxide H_2O_2 according to the method of Wolf (1982). Na^+, K^+ and Ca^{2+}cations were determined with a flame photometer. Cl^- anion was determined with water distillation. The samples were placed into oven at 80ºC for 7-8 h. After filtering, Cl^- was measured with a Chloride meter.

The experiment was laid out in a Completely Randomized Block Design (CRBD) and analysis of variance technique was employed for carrying out statistical analysis of data collected (Steel and Torrie, 1980). Various treatment means were compared with Duncan's New Multiple Range (DMR) Test.

RESULTS AND DISCUSSION

Salinity had highly significant effect on shoot length in black seeds. Shoot length decreased with the increase in salinity level. Maximum reduction 51.9% of control was observed at 6 dS/m while at 3 dS/m the reduction was observed 33.22% of control. In control shoot length was 12.58 cm while at 3 and 6 dS/m it reduced to 8.4 and 6.05 cm respectively (Table 1). Salinity significantly increased the root length in black seeds (Fig. 2). In control root length was observed 3.45 cm while at 3 and 6 dS/m it increased 4.33 and 5.13 cm respectively (Table 1). These results are similar with the earlier findings in wheat (Sharma, 1995).

Fig. 3 shows that shoot fresh and dry weights in black seeds decreased with the increase in salinity levels. Shoot fresh weight decreased 52.48% at 6 dS/m and 28.46% at 3 dS/m from control. Shoot fresh weight was 21.15 g in control while at 3 and 6 dS/m it decreased to 15.13 and 10.05 g respectively (Table 1). Shoot dry weight decreased to 44.71 and 27.35% at 6 dS/m and 3 dS/m respectively from control.

It was 5.31 g in control while at 3 and 6 dS/m shoot dry weight was observed 3.85 and 2.93 g respectively (Table 1). These results are similar with the earlier findings in maize (Izzo *et al.*, 1996).

Na^+ concentrations increased with the increase in salinity levels in black seeds. Na^+concentration increased to 11.17 mg/g in plants having 6 dS/m and 8.52 mg/g at 3 dS/m. In contrast in control the Na^+ concentration was 4.42 mg/g (Table 2). Similar results have been found in a number of studies as in wheat (Begum *et al.*, 1992).

Table,2 shows that the effect of salinity was significant on K^+ concentrations. K^+ concentrations increased with the increase in salinity levels. K+ concentration was higher in plants having 6 dS/m NaCl that was 15.75 mg /g while at 3 dS/m. These results are with agreement what has earlier been found by Izzo *et al.* (1996).

Data regarding Ca^{2+} concentration shows that the effect of salinity was highly significant in black seeds (Table 2). Ca^{2+} concentration decreased under both levels of salinity (3 and 6 dS/m) that was 4 and 4.47 mg/g respectively (Table 2). Reduction in $Ca^{2+}$was observed in black seeds, these results are similar with Ashraf and Khanum (1997) in wheat.

NaCl had a highly significant effect on Cl^- concentration in black seeds. Cl^- increased with the increase in salinity levels. It was 13.0 mg/g in plants treated with 6 dS/m salinity while in control and at 3 dS/m it was 5.82 and 8.97 mg/g (Table 2). Similar results have been found similar as in *Hibiscus cannabinus* (Francois *et al.*, 1992).

Fig 1: Effect of different salinity levels on growth of black seeds

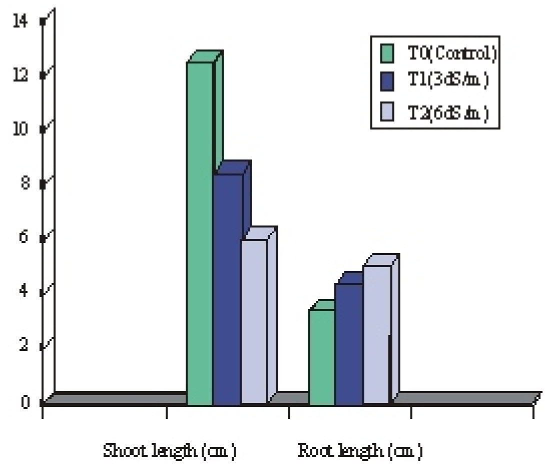

Fig 2: Shoot and root length (cm) in black seeds under different salinity levels

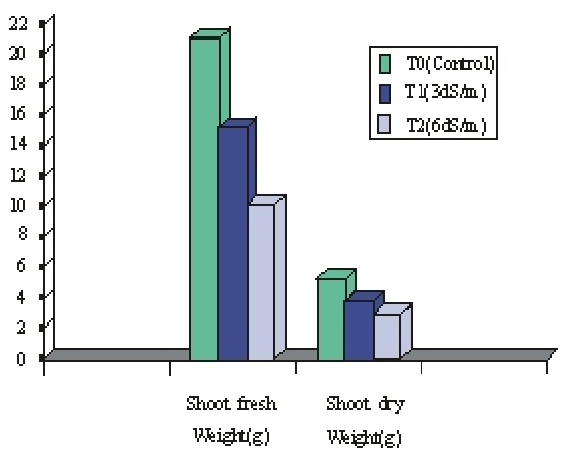

Fig 3: Shoot fresh and dry weight (g) in black seeds under different salinity levels

Addition of NaCl had an adverse effect on the growth of black seeds. Salinity caused a significant effect on shoot and root lengths, shoot fresh and dry weights. The reason for growth reduction in black seeds could be due to water shortage and ionic toxicity caused by salinity. The increase in plant growth may be due to turgor potential which is decreased by water deficit produced by high concentrations of the salts in the soil (Ashraf and Naqvi, 1996). Assessment of pattern of accumulation of toxic ions in a species is vital importance to understand, whether the species uses partial exclusion or inclusion mechanism for tolerating toxic ions present in its growth medium (Reggiani *et al*., 1995).

Table 1: Effect of different salinity levels on growth of black seeds

Parameters	0 dS/m	3 dS/m	6 dS/m	LSD values
Shoot length (cm)	12.58a	8.4b	6.05c	1.98
Root length (cm)	3.45b	4.33ab	5.13a	1.01
Shoot fresh weight (g)	21.15a	15.13b	10.05c	2.25
Shoot dry weight (g)	5.31a	3.85b	2.93c	0.95

Mean values followed by different small letter indicates difference among NaCl treatments at $P > 0.05$

Table 2: Effect of different salinity levels on ion contents of black seeds

Concentrations	0 dS/m	3 dS/m	6 dS/m	LSD values
Na^+ (mg/g)	4.42c	8.52b	11.17a	1.87
K+ (mg/g)	7.72c	12.24b	15.75a	2.24
Ca^{2+} (mg/g)	6.5a	4.47b	4.0b	0.78
Cl^- (mg/g)	5.82a	8.97b	13.0c	1.32

Mean values followed by different small letter indicates difference among NaCl treatments at $P > 0.05$

It is concluded that salinity had adverse effect on growth in black seeds. Growth reduced with the increase in salinity levels. Ion contents also varied significantly under salt stress. So, salinity had adverse effects on plant life cycle.

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