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The effects of saline irrigation water and cobalt on growth and chemical composition in *Nigella sativa*

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Abstract. *Khalid KA, Shedeed MR. 2014. The effects of saline irrigation water and cobalt on growth and chemical composition in* Nigella sativa*. Nusantara Bioscience 6: 146-151.* Increasing plant salinity tolerance is a focus of research and industry since salinity and yield are of major concern to maximize medicinal and aromatic plant production in arid and semi-arid areas. Therefore, the present study aimed to decrease the harmful effect of salinity on *Nigella sativa* L plants by adapting them to saline soil stress through the use of Cobalt. The effects of saline irrigation water and cobalt on the vegetative growth characters [plant height (cm), leaf number (plant^{-1}), branch number (plant^{-1}), capsule number (plant^{1}), herb dry weight (plant^{-1}) and seed yield (plant^{-1})] and content of fixed oil, soluble sugars, proline, N,P,K and protein of black seed (*Nigella sativa* L.) plants were investigated. In these experiments, two factors were considered: saline irrigation water and Cobalt. The experimental design followed a complete random block design. The averages of data were statistically analyzed using 2-way analysis of variance (ANOVA-2) and the values of least significant difference (LSD) at 5%. Saline irrigation water decreased certain growth characters, fixed oil, protein and mineral content (N, P and K) as saline irrigation water level increased. Saline irrigation water promoted the accumulation of soluble sugars and proline contents. The plants treated with saline irrigation water containing cobalt resulted in higher plant growth characters and chemical constituent's values than those treated with saline irrigation water alone.

Key words: Biochemical contents, cobalt, growth characters, *Nigella sativa*, saline irrigation water

INTRODUCTION

The black seed, *Nigella sativa* L., a member of the family of Ranunculaceae, contains more than 30% of fixed oil and 0.4-0.5% of volatile oil (VO). The VO contains 18-24% thymoquinone and 46% monoterpenes such as *p*-cymene and α-pinene (El-Kadi and Kandil 1987). Recently, clinical and animal studies have shown that extract of the black seeds have many therapeutic effects such as immunomodilative (Hanafy and Hatem 1991), antibacterial (Zaoui et al. 2000), hypotensive (Turkdogan et al. 2001), hepatoprotective (Kanter 2003) and antidiabetic effects (Houghton 1995). Ohkawa et al. (1978) also reported that black seed oil and its derivative thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation.

Saline soil can be defined as soil having an electrical conductivity of the saturated paste extract (ECe) of 4 dS m^{-1} (4 dS m^{-1} ~ 40 mM NaCl) or more. Salinity is a major factor reducing plant growth and productivity worldwide; it affects about 7% of the world's total land area (Flowers 1997; Zhu 2002) and is the major environmental factor limiting plant growth and productivity (Allakhverdiev et al. 2000). The detrimental effects of high salinity on plants can be observed at the whole plant level such as the death of plants or necrosis of plant organs and/or decreases in productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within cells (Kobayashi 2008). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, energy, lipid metabolism and hormonal balance are affected. The earliest response is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies. Growth often resumes when the stress is relieved. Carbohydrates, which among other substrates are needed for cell growth, are supplied mainly through the process of photosynthesis, and photosynthetic rates are usually lower in plants exposed to salinity, especially to NaCl (Parida and Das 2005). Plants subjected to saline conditions after the early seeding stage rapidly resumed normal growth rate when the stress was removed but opposite trend was found for the plants subjected to stress during the early seeding stage (El-Gamal 2000). Hanafy et al. (1994) reported that salinity levels (up to 6000 ppm) decreased certain growth characters (such as plant height, plant dry weight, seed yield), N, K and protein content of black seed plant; while total sugars increased as salinity level increase. Kotb and El-Gamal (1994) showed that salinity level up to 0.3% had a significant decrease in seed germination, branch number, dry weight, seed yield, fixed oil and total carbohydrate of black seed plants. Shoot and root dry weights, soluble and soluble protein decreased as NaCl level increased. Salinity promoted the accumulation of total carbohydrates, proline in of black seed (Hajar et al. 1996). Salinity stress decreased fresh and dry weight of *Calendula officinalis* L. flowers (Khalid and da Silva

2010). Salinity decreased certain growth characters and mineral content of lemon balm (Khalid and Cai 2011).

There was a significant effect of the interaction between NaCl salinity and cobalt (Co) levels on the dry matter yields of shoots, roots and whole tomato plant (El-Gamal 2000). Moreno-Caselles (1997) reported that total chlorophyll contents were affected by Co level in tomato plants. The effects of Co on seedling vigor, biochemical constituents and mineral status in *Raphanus sativus* L. were studied by Jayakumar et al. (2007). The plants were raised in pots containing soils amended with different concentrations of Co (50, 100, 150, 200, and 250 mg kg^{-1}). Growth parameters, and biochemical constituents (total sugar, amino acid, and protein content), mineral content, were analyzed. Measures increased at the 50 mg Co kg^{-1} soil level when compared to the control. Further increases in the Co level (100-250 mg kg^{-1} soil) had a negative effect on these parameters. Gad and Kandil (2010) revealed that Co treatments caused a significant increase of nitrogen (N), phosphorus (P) but no significant increase was observed in potassium (K) content of tomato plants. Co addition enhanced the growth characters and yield of tomato plant. Aziz et al. (2011) indicated that Co at 15 ppm caused a significant increase in fresh and dry herb, essential oil content and macro-nutrient content (N, P and K) of peppermint **(***Mentha* x *piperita***).**

Increasing plant salinity tolerance is a focus of research and industry since salinity and yield are of major concern to maximize medicinal and aromatic plant production in arid and semi-arid areas. Therefore, the present study aimed to decrease the harmful effect of salinity on *Nigella sativa* L plants by adapting them to saline soil stress through the use of Cobalt.

MATERIALS AND METHODS

Experimental

The present study was carried out in the Experimental Farm, Faculty of Agriculture, Ain Shams University, located at Shubra El-Kheima, Kalubia, Egypt, during two successive seasons of 2006/2007 and 2007/2008. *Nigella sativa* L seeds were obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. In the first week of November during both seasons seeds were sown in plastic pots (30cm diameter and 50cm height), 10 seeds per pot. The viability of seeds was approximately 92%. In the third week of December during both seasons, the pots were transferred to a greenhouse adjusted to natural conditions. Each pot was filled with 10 kg of air-dried clay loam soil. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie et al. (1982) and are presented in Table 1. Eight weeks after sowing the seedlings were thinned to three plants per pot. Pots were divided into four main groups. The first group was subjected to different levels of saline irrigation water, 0.39 (tap water as control), 1.56, 3.13, 4.69 and 6.25 dSm^{-1}. To prepare irrigation water with different salinity levels, highly soluble NaCl salt was used. The second, third and fourth groups were subjected to the treatments of saline irrigation water but Co was added at 25, 50 and 75 ppm respectively. All agricultural practices were conducted according to the main recommendations by the Egyptian Ministry of Agriculture.

Harvesting

At fruiting stage, the plants were harvested at the end of the two seasons. Vegetative growth characters measurements were recorded, namely: plant height (cm), leaf number ($plant^{-1}$), branch number ($plant^{-1}$), capsule number ($plant^{1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$).

Total soluble sugars (TSS) determination

TSS concentrations in seeds (collected at the end of the first and second season of each treatment) were determined according to Ciha and Brun (1978) with some modifications. Samples of 100 mg were homogenized with 10 mL of extracting solution [glacial acetic acid: methanol: water, 1:4:5, v/v/v. The homogenate was centrifuged for 10 min at 3.000 rpm and the supernatant was decanted. The residue was re-suspended in 10 mL of extracting solution and centrifuged another 5 min at 3.000 rpm. The supernatant was decanted, combined with the original extract and made up to 50 mL with water. For measurement of total carbohydrates and TSS, a phenol-sulfuric acid assay was used (Dubois et al. 1956). A volume of 0.5 mL of 5% (v/v) phenol solution and 2.5 mL of concentrated sulfuric acid were added to 0.5 mL aliquots. The mixture was shaken, heated in a boiling water-bath for 20 min and cooled to room temperature. The absorption was then determined by spectrophotometer at 490 nm.

Fixed oil (FO), nutrients and protein determination

FO extraction: 50 g of seeds were crushed to coarse powered and extracted with petroleum ether (40-60 ^{o}C) in a Soxhlet apparatus (AOAC 1970). N, protein, P and K (in the leaves) of both seasons of each treatment were determined using the methods described by the AOAC (1970) as follows: The washed and dried materials were ground to fine powder with mortar and pestle and used for dried ashing. For analysis of K the powdered plant material (0.2 g) was taken in precleaner and constantly weighed silica crucible and heated in muffle furnace at 400 ^{0}C till there was no evolution of smoke. The crucible was cooled in desiccator at room temperature. The ash totally free from carbon moistened with Conc. H_2SO_4 and heated on Hot plate till fumes of sulphuric acid get evolved the silica crucible with sulphated ash was again heated at 600 ^{0}C in muffle furnace till weight of sample was constant (3-4 hrs) one gram sulphated ash were taken in beaker which dissolved in 100 mL 5% conc. HCl to obtain solution for determination of K through flame photometry, standard solution of each mineral was prepared and calibration curve drawn for K element using flame photometry. For determination of protein and Nitrogen using Micro Kjeldahl method, 1 g of plant sample taken in a Pyrex digestion tube and 30 mL of conc. H_2SO_4 carefully added,

then 10 g potassium sulphate and 14 gm copper sulphate, mixture is placed on sand both on a low flame just to boil the solution, it was further heated till the solution becomes colorless and clear, allowed to cool, diluted with distilled water and transferred 800 mL Kjeldahl flask, washing the digestion flask, Three or four pieces of granulated zinc and 100 mL of 40% caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 mL of 0.1 N sulphuric acids was taken in the receiving flask and distilled; it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using Methyl Red indicator for determination of nitrogen, which in turn give the protein content. For determination of phosphorous 2 g sample of plant material taken in 100 mL conical flask two spoons of Darco-G-60 is added followed by 50 mL of 0.5 M NaHCo3 solution, next flask was corked, and allowed for shaking for 30 min on shaker. the content was filtered and filtrate was collected in flask from which 5 mL filtrate was taken in 25 mL volumetric flask to this 2 drops of 2, 4-paranitrophenol and 5 N H_2SO_4 drop by drop was added with intermittent shaking till yellow color disappear, content was diluted about 20 mL with distilled water and then 4 mL ascorbic acid was added then the mixture was shacked well and the intensity of blue color at 660 nm on colorimeter was measured. The absorbencies were compared and concentrations of phosphorous using standard value were calculated.

Proline determination

Proline was determined at vegetative, flowering and fruiting stages in leaves according to Bates (1973) as follows: Samples:-Fully expanded (sun) leaves from field-grown black cumin plants were sampled; purified proline was used to standardize the procedure for quantifying sample values. Reagents:-Acid-ninhydrin was prepared by warming 1.25 gm ninhydrin in 30 mL glacial acetic and 20 mL, 6 m phosphoric acid with agitation until dissolved. Kept cool (stored at 4C°) the reagent remains stable 24 hours (Procedure. 1). Approximately 0.5gm of plant material was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatmann # 2 filter paper. 2). Two mL of filtrate was reacted with 2 mL acid ninhydrin and 2 mL of glacial acetic acid in test tube for 1 hours at 100 C°, and the reaction terminated in an ice bath. 3.). The reaction mixture was extracted with 4 mL toluene, mixed vigorously with test tube stirrer 15-20 sec. 4). The chromophore containing toluene was a spirated from aqueous phase, warmed to room temperature and the absorbance read at 520 n.m. using to standard curve and calculated on a fresh weight basis as follow: -(µg proline/mL.× mL toluene/15.5 µg/µmol) {(g sample)/5}= µmoles proline/g of fresh weight material.

Statistical analysis

In these experiments, two factors were considered: saline irrigation water and Cobalt. For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual plants. The experimental design followed a complete random block design. According to Snedecor and Cochran (1990), the averages of data were statistically analyzed using 2-way analysis of variance (ANOVA-2) and the values of least significant difference (LSD) at 5%.

RESULTS AND DISCUSSION

Growth characters (GC)

Saline irrigation water (SIW) and Co affected plant morphology inboth the first and second seasons (Table 2). GC such as plant height (cm), leaf number ($plant^{-1}$), branch number ($plant^{-1}$), capsule number ($plant^{1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$) were affected by changes in saline irrigation water with or without Co. Thus the various GC in general decreased under the various SIW, especially at 6.3 dSm^{-1} treatment. Greatest yields at each season for all variables were obtained in the 0.4 dSm^{-1}+cobalt at 50 mg L^{-1} treatment (Table 2).

ANOVA indicated that the changes in GC were significant for SIW, Co and their interactions. The inhibition of plant growth characters under SIW treatments may be due to exposure to injurious levels of salinity during both seasons causing a decrease of turgor which would result in a decrease of growth and development of cells, especially in stems and leaves (Merrill and Eckard 1971). Cell growth is the most important process and is affected by salinity stress. Plant size is indicated by a decrease in height or smaller size of leaves when there is a decrease in the growth of cells (Hsiao 2000).When leaf size is smaller, the capacity to trap light decreases too and the capacity of total photosynthesis decreases, i.e. photo-synthesis is restricted in salt stress conditions, with a

Table 1. Physical and chemical properties of the soil (average of 3 samples from 30-50 cm depth)

Clay (%)	Silt (%)	Sand (%)	Texture
67.0	9.0	24.0	Clay

Soluble cations (mg/100 soil)				Soluble anions (mg/100 soil)				
Ca	Mg	Na	K	Co_3	HCO_3	Cl	SO_4^-	
106.0	62.0	41.0	39.8	-	2.0	5.0	-	106.0

OM (%)	SP (%)	$CaCo_3$ (%)	pH	EC ($dS\ m^{-1}$)	NO_3 (ppm)	P (ppm)	CO ($mg\ L^{-1}$)	SAR
1.4	31.8	4.8	7.2	1.8	20.1	1.5	-	4.5

Note: OM = Organic Matter, SP= Saturation Percentage, EC= Electronic Conductivity, SAR= Sodium Adsopation Ratio.

Table 2. Effect of saline irrigation water, cobalt and their interactions on growth characters

Treatments		Plant height (Cm)		Leaf number (Plant-^{1})		Branch number (Plant-^{1})		Capsule number (Plant-^{1})		Dry weight (g Plant-^{1})		Seed yield (g Plant-^{1})	
Cobalt (mg L^{-1})	dSm^{-1}	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0	0.4	9.3	11.3	28.4	27.6	3.0	3.6	2.5	2.6	15.2	18.6	3.8	4.3
	1.6	8.6	9.7	26.4	25.2	2.6	3.1	2.5	2.4	14.0	14.7	3.0	3.4
	3.1	5.6	8.3	25.0	22.7	2.0	2.6	2.2	2.2	11.4	11.2	2.7	3.3
	4.7	3.6	6.7	19.0	21.1	1.4	2.3	2.2	1.9	9.0	10.2	2.2	3.0
	6.3	3.1	4.1	5.7	5.8	1.2	2.2	2.0	2.2	4.8	5.8	2.1	1.9
Overall 0		6.0	8.0	20.9	20.5	2.0	2.8	2.3	2.3	10.9	12.1	2.8	2.2
25	0.4	15.6	19.5	49.7	39.6	5.0	4.0	11.0	8.3	23.3	22.1	4.2	5.1
	1.6	13.7	16.3	46.7	35.3	4.8	3.9	7.7	6.3	22.0	20.6	4.0	4.8
	3.1	11.7	13.0	45.2	31.9	4.4	3.3	3.2	4.2	20.2	19.2	3.9	4.3
	4.7	9.6	11.3	29.4	29.5	3.8	2.8	2.6	2.7	16.3	16.2	3.8	4.2
	6.3	5.4	7.7	10.8	10.8	1.7	2.4	2.4	2.5	7.0	9.3	3.0	2.0
Overall 25		11.2	13.6	36.4	29.4	3.9	3.3	5.4	4.8	17.7	17.5	3.8	4.1
50	0.4	18.8	34.3	58.7	58.5	6.0	7.4	15.8	14.5	30.3	31.2	4.4	5.7
	1.6	16.8	26.4	57.9	44.3	5.6	6.3	13.0	12.1	28.1	29.9	4.3	5.1
	3.1	14.9	20.2	57.1	41.2	5.2	4.9	12.0	10.4	26.1	27.6	4.1	4.6
	4.7	12.8	15.6	48.0	37.0	4.8	4.4	11.2	8.3	24.6	25.0	4.0	4.5
	6.3	10.8	12.4	31.7	14.2	4.0	3.8	6.2	4.5	21.9	22.2	3.9	4.3
Overall 50		14.8	21.8	50.7	39.0	5.1	5.4	11.6	10.0	26.2	27.2	4.1	4.8
75	0.4	9.9	12.9	48.6	39.3	3.4	4.5	5.27	7.8	11.9	13.6	3.1	4.1
	1.6	9.1	11.2	47.2	30.7	2.8	2.9	4.03	4.8	9.4	11.4	3.0	3.9
	3.1	7.8	10.6	41.3	25.2	2.0	2.5	3.23	3.9	8.5	10.0	2.9	3.7
	4.7	6.8	9.0	22.2	23.5	1.2	1.6	1.84	3.1	7.7	8.7	2.6	3.3
	6.3	3.5	6.8	15.9	12.9	2.1	1.9	2.0	3.0	7.5	8.5	2.0	2.1
Overall 75		7.4	10.1	35.0	26.3	2.3	2.7	3.3	4.5	9.0	10.4	2.7	3.4
Overall	0.4	13.4	19.5	46.4	41.3	4.4	4.9	8.6	8.3	20.2	21.4	3.9	4.8
salinity	1.6	12.1	15.9	44.6	33.9	4.0	4.1	6.8	6.4	18.4	19.2	3.6	4.3
treatments	3.1	10.0	13.0	42.2	30.3	3.4	3.3	5.2	5.2	16.6	17.0	3.4	4.0
	4.7	8.2	10.7	29.7	27.8	2.8	2.8	4.5	4.0	14.4	15.0	3.2	3.8
	6.3	5.7	7.8	16.0	10.9	2.3	2.6	3.2	3.1	10.3	11.5	2.8	2.6
LSD: 0.05													
Salinity		0.7	1.0	2.3	3.3	0.5	0.4	1.1	0.6	3.5	3.1	0.3	0.6
Cobalt		0.6	0.9	4.5	4.8	0.4	0.3	1.0	0.5	2.1	2.2	0.2	0.5
Salinity * Cobalt		1.3	2.0	5.6	5.7	1.1	0.7	2.2	1.2	4.1	3.7	0.4	1.1

Note: 1st = first season, 2nd = second season

Table 3. Effect of saline irrigation water, cobalt and their interactions on chemical constituents

Treatments		Fixed oil (%)		Proline (µm/g)		Soluble sugars (%)		Protein (%)		N (%)		P (%)		K (%)	
Cobalt (mg L^{-1})	dSm^{-1}	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0	0.4	16.8	18.2	5.5	4.9	5.3	2.6	17.5	16.0	2.8	2.6	0.3	0.4	0.8	1.0
	1.6	16.1	17.6	8.9	9.0	8.0	4.6	15.6	15.0	2.5	2.4	0.2	0.3	0.6	0.8
	3.1	15.6	16.2	10.5	11.0	9.0	6.0	13.8	13.8	2.2	2.2	0.2	0.2	0.5	0.4
	4.7	14.1	15.1	15.1	13.0	11.0	6.4	12.5	10.0	2.0	1.6	0.1	0.2	0.3	0.2
	6.3	14.0	13.2	21.4	17.0	12.8	7.5	7.5	5.6	1.2	0.9	0.1	0.1	0.2	0.2
Overall 0		15.3	16.1	12.3	11.0	9.2	5.4	13.4	12.1	2.1	1.9	0.2	0.2	0.5	0.5
25	0.4	24.7	21.6	6.2	4.8	8.3	3.3	16.3	16.9	2.6	2.7	0.4	0.5	0.6	0.6
	1.6	20.2	21.0	12.9	9.0	10.0	5.3	15.3	15.6	2.4	2.5	0.4	0.4	0.5	0.4
	3.1	17.3	18.2	16.1	11.0	10.5	6.5	15.0	15.0	2.4	2.4	0.3	0.3	0.4	0.4
	4.7	16.2	14.9	19.7	16.0	13.1	9.0	12.5	13.1	2.0	2.1	0.3	0.2	0.2	0.3
	6.3	15.0	13.5	24.6	22.0	14.5	9.7	8.1	8.8	1.3	1.4	0.2	0.2	0.2	0.2
Overall 25		18.7	17.7	15.9	12.6	11.3	6.8	13.5	13.9	2.2	2.2	0.3	0.3	0.4	0.4
50	0.4	25.1	25.9	12.7	16.2	12.8	3.4	17.5	19.3	2.8	3.1	0.8	0.9	0.8	1.1
	1.6	24.3	25.3	23.3	14.8	14.5	5.7	16.9	18.1	2.7	2.9	0.7	0.8	0.7	0.9
	3.1	22.8	24.1	24.3	19.3	15.9	7.5	16.3	16.9	2.6	2.7	0.6	0.8	0.6	0.5
	4.7	22.3	22.5	25.2	21.9	18.0	10.5	15.0	16.3	2.4	2.6	0.6	0.8	0.3	0.5
	6.3	21.1	21.2	31.7	24.5	20.9	11.2	14.4	15.0	2.3	2.4	0.4	0.7	0.3	0.4
Overall 50		23.1	23.8	23.4	19.3	16.4	7.7	16.0	17.1	2.6	2.7	0.6	0.8	0.5	0.7
75	0.4	18.8	23.1	6.1	5.8	4.5	2.6	14.4	13.8	2.3	2.2	0.4	0.5	0.5	0.3
	1.6	17.1	22.2	12.3	12.0	6.0	4.6	13.8	11.9	2.2	1.9	0.3	0.4	0.3	0.3
	3.1	16.6	20.0	14.1	14.8	7.3	6.0	12.5	11.3	2.0	1.8	0.2	0.3	0.2	0.2
	4.7	14.3	17.0	20.4	18.0	9.3	6.4	11.3	9.4	1.8	1.5	0.1	0.3	0.1	0.2
	6.3	16.8	15.8	22.2	20.9	9.8	7.5	8.8	8.8	1.4	1.4	0.1	0.3	0.1	0.2
Overall 75		16.7	19.6	15.0	14.3	7.4	5.4	12.2	11.0	1.9	1.8	0.2	0.4	0.2	0.2
Overall	0.4	21.4	22.2	7.6	7.9	7.7	3.0	16.4	16.5	2.6	2.6	0.5	0.6	0.7	0.7
salinity	1.6	19.4	21.5	14.4	11.2	9.6	5.1	15.4	15.2	2.5	2.4	0.4	0.5	0.5	0.6
treatments	3.1	18.1	19.6	16.3	14.0	10.7	6.5	14.4	14.3	2.3	2.3	0.3	0.4	0.4	0.4
	4.7	16.7	17.4	20.1	17.2	12.9	8.1	12.8	12.2	2.1	2.0	0.3	0.4	0.2	0.3
	6.3	16.7	15.9	25.0	21.1	14.5	9.0	9.7	9.6	1.6	1.5	0.2	0.3	0.2	0.3
LSD															
Salinity		0.6	0.4	1.1	1.5	0.6	0.4	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Cobalt		0.5	0.3	0.9	1.0	0.5	0.4	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Salinity * Cobalt		1.2	1.0	2.1	1.3	1.1	0.6	0.5	0.6	0.1	0.1	0.1	0.1	0.1	0.2

Note: 1st = first season, 2nd = second season

subsequent reduction in plant growth and performance (Hsiao 2000). Salinity stress resulted in significant reductions in CO_2 exchange rate, total assimilatory area, fresh and dry matter in Japanese mint (*Mentha arvensis* L. cv. MS 77) (Misra and Srivastava 2000). The loss of photosynthesis in salinity stress conditions results in a loss of dry matter production at the leaf level of mungbean, bean, topiary bean, *Sesuvium portulacastrum* (ambiguously) and *Pesquisa agropecuaria* (Embrapa) plants (Cox and Jolhff 1987; Viera et al. 1991; Slama 2007). The increases in growth characters under cobalt or cobalt + saline irrigation water treatments may be due to an increase in chlorophyll content caused by Co, and consequently, photosynthesis efficiency; on the other hand, Co increases the total carbohydrates (source of energy) and mineral content, so that plant growth parameters increased under cobalt or Co + SIW treatments (Aziz et al. 2011).

Fixed oil (FO)

As shown in Table 3, FO content decreased at all SIW treatments. Co or SIW + Co levels treatments increased the FO accumulation during both seasons. The highest accumulation of FO was recorded at the lowest SIW level (control) x Co at 50 mg L^{-1} interaction compared with other treatments during the first and second season. ANOVA indicated that the changes in FO were significant for SIW, Co and their interactions. The effect of different treatments (SIW, Co and their interactions) on FO may be due to its effect on enzyme activity and metabolism of fixed oil production (Burbott and Loomis 1969).

Total soluble sugars (TSS)

TSS content increased with saline irrigation water, Co and the Co ×SIW interaction in both seasons (Table 3). However, the highest TSS content resulted from 6.3 dSm^{-1} + Co at 50 mg L^{-1} treatment compared with control for the first and second seasons. ANOVA indicated that the changes in TSS were significant for SIW, Co and their interactions. In this study, the SIW, Co and the Co ×SIW interaction treatments enhanced the plant to preserve TSS for sustained metabolism, prolonged energy supply, and for better recovery after stress relief (Hanafy et al. 1994; Moreno-Caselles 1997; Hendawy and Khalid 2005; Slama et al. 2007).

Proline

The accumulation of proline in *N. sativa* leaves during the first and second seasons was promoted by applying various levels of SIW, Co and their interactions (Table 3). The highest proline content resulted from 6.3 dSm^{-1} with 50 mg L^{-1} Co treatment. ANOVA indicated that the changes in proline were significant for SIW, Co and their interactions. These results agree with those of (Blum and Ebercon 1976; Moreno-Caselles 1997; Slama et al. 2007) who indicated that proline is regarded as a source of energy, carbon, and nitrogen for recovering tissues under saline irrigation water and/or cobalt.

N (protein) PK

Increase in SIW level caused a decrease in measured protein and nutrient content such as macro elements (N, P and K) (Table 3). Addition of Co ameliorated this decline with increasing salt stress. SIW at 6.3 dSm^{-1}resulted in the lowest nutrient and protein accumulations while the highest protein and mineral contents were observed in the control treatment + 50 mg L^{-1} of Co. ANOVA indicated that the changes in N (protein), P and K were significant for SIW, Co and their interactions. The loss of nutrients and protein under salinity stress is probably due to less availability of these elements to plants (El-Sherif et al. 1990). Co or Co + SIW treatments increased the concentrations of protein and nutrients. Also El-Sherif et al. (1990) and Kaliyamoorthy et al. (2007) reported that trace elements or Co caused an increase in the accumulation of protein and nutrients in tomato and Radish plants.

CONCLUSION

The plants treated with saline irrigation water with Cobalt resulted in higher growth characters and chemical constituent's values than those treated with SIW alone. It may be concluded that Cobalt reduces the harmful effect of salt stress for black seed (*Nigella sativa* L.) plants.

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