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Seed Yield, Fixed Oil, Fatty Acids and Nutrient Content of *Nigella sativa* L. Cultivated under Salt Stress Conditions

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ABSTRACT
Increasing plant salinity tolerance is a focus of research and industry since salinity and yield are of major concern to maximize medicinal and aromatic plants production in arid and semi-arid areas. In this study, we investigate the possible effect of salinity on the seed yield, fixed oil; fatty acids and nutrient content (NPK) of *Nigella sativa* L an important medicinal plant. *Nigella sativa* is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine. Plants were subjected to different levels of saline irrigation water, 0.39 (tap water as control), 1.56, 3.13, 4.69 and 6.25 dS m^{-1}. The averages of data were statistically analyzed using one-way analysis of variance (ANOVA-1). Significant values determined according to p values ($p<0.05$ = significant, $p<0.01$ = moderate significant and $p<0.001$ = highly significant). The seed yield, fixed oil and nutrient content (NPK) in general decreased under the various saline treatments. Linoleic, Oleic and Stearic were detected as the major fatty acids of the fixed oil in all the treatments which changed under different salinity levels.

Key words: *Nigella sativa* L, seed yield, fixed oil, fatty acids, NPK

INTRODUCTION

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. In this study, we investigate the possible effect of saline irrigation water on the seed yield, fixed oil, fatty acids and nutrient content (NPK) of *Nigella sativa* L. an important medicinal plant.

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 *et al*., 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 (Parida and Das, 2005). The earliest response is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies (Parida and Das, 2005). 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 and seed yield), N, K and protein content of *Nigella sativa* L. 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 *Nigella sativa* L. plants. Shoot and root dry weights, soluble and insoluble protein decreased as NaCl level increased. Salinity promoted the accumulation of total carbohydrates, proline of *Nigella sativa* L. (Hajar *et al*., 1996). *Nigella sativa* L. seed yield, oil yield and water use efficiency based on seed and oil yields decreased rapidly as a result of increased of water salinity during irrigation (Ghamarnia *et al*., 2012). Salinity stress decreased fresh and dry weight of *Calendula officinalis* L. flowers and sage plant (Hendawy and Khalid, 2005; Khalid and da Silva, 2010). Salinity decreased certain growth characters and mineral content of lemon balm (Khalid and Cai, 2011).

Nigella sativa (Family Ranunculaceae) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *N. sativa* have been widely used in the treatment of different diseases and ailments. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Prophetic medicine. It has been widely used as antihypertensive, liver tonics, diuretics, digestive, antidiarrheal, appetite stimulant, analgesics, antibacterial and in skin disorders (Ahmad *et al*., 2013).

MATERIALS AND METHODS

Experimental: The present study was carried out in the Experimental site, National Research Centre (NRC), located at Giza area, Egypt, during two successive seasons of 2010/2011. *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 seeds were sown in plastic pots (30 cm diameter and 50 cm height), 10 seeds per pot. The viability of seeds was approximately 92%. In the third week of December, 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. Plants were subjected to different levels of saline irrigation water, 0.39 (tap water as control), 1.56, 3.13, 4.69 and 6.25 dS m^{-1}. To prepare irrigation water with different salinity levels, highly soluble NaCl salt was used. All agricultural practices were conducted according to the main recommendations by the Egyptian Ministry of Agriculture.

Table 1: Physical and chemical properties of the soil

Properties	Values
Clay (%)	24
Silt (%)	9
Sand	67
Texture	Clay
Soluble cations (mg/100 soil)	
Ca	106
Mg	62
Na	41
K	40
Soluble anions (mg/100 soil)	
CO_3	-
HCO_3	2
Cl	5
SO_4	106
Organic matter (%)	1.4
Saturation percentage	32
$CaCO_3$ (%)	4.8
pH	7.2
Electronic conductivity (dS m^{-1})	1.8
NO_3 (ppm)	20.1
P (ppm)	1.5
CO	-
Sodium adsorption ratio	4.5

Harvesting: At fruiting stage, the plants were harvested at the end of the two seasons. Seed yields (Plant^{-1}) were recorded.

Extraction of fixed oil: The seeds (50 g) were powdered mechanically and extracted with light petroleum ether (40-60°C) for 4 h in a Soxhlet apparatus. Removal of the solvent under reduced pressure gave the fixed oils (AOAC., 1970).

Gas chromatography: The fatty acid content of the fix ed oils was investigated by GC analysis of their methyl esters. Oil (0.5 g) was dissolved in 20 mL light petroleum ether (60-80°C) and 2 mL 2 M methanolic KOH was added. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper layer was removed, washed with water and 1 mL used for GC analysis (Houghton *et al*., 1995).

The GC analyses were performed using an HP 6890 gas chromatograph with a Supelco SP23 80 capillary column (60 m×0.25 mm×0.20 µm) and helium as the carrier gas. The oven temperature was kept at 140°C for 5 min, programmed to 165°C at a rate of 5°C min^{-1} and kept at 165°C for 10 min, then program med to 190°C at a rate of 5°C min^{-1} and kept at 190°C for 20 min. Injector and detector (FID) temperatures were k pt at 250°C. The split ratio was 70:1. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

Gas chromatography mass spectrometry (GC-MS): The GC-MS analyses of the oils were carried out on an HP GC-MS 6890-5 973 model instruments. The GC column used was a Supelco SP23 80 capillary column (60 m×0.25 mm×0.20 µm). Oven temperature was as above, transfer line temperature

280°C, ion source temperature 230°C, carrier gas helium; splitting ratio 1:10, ionization energy 70 eV, scan range 15-550 amu.

Qualitative and quantitative analyses: Compounds were identified by comparison of their GC retention times with those of reference solutions of 1% w/v of the methyl esters of the fatty acid and also by comparison of their mass spectra with either known compounds or published spectra (Wiley 275 L). Quantification of fatty acid methylesters was obtained directly from GC peak area using Chemstation 8.02 software and expressed as percent ages.

Nutrient determination: The N, P and K of both seasons of each treatment were determined using the methods described by AOAC (1970) as follows: The washed and dried materials were ground to fine powder with mortar and pestle and used for dried ash. For analysis of K the powdered plant material (0.2 g) was taken in precleaned and constantly weighed silica crucible and heated in muffle furnace at 400°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 sulfated ash was again heated at 600°C in muffle furnace till weight of sample was constant (3-4 h) one gram sulfated ash were taken in beaker which dissolved in 100 mL 5% conc. The 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 g 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 M 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 M 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 $NaHCO_3$ 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 M 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.

Statistical analysis: In these experiments, one factor was considered: Saline irrigation water. 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 one-way analysis of variance (ANOVA-1). Significant values determined according to p values ($p<0.05$ = significant, $p<0.01$ = moderate significant and $p<0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

RESULTS

Effect of salinity on seed yield: Data in Table 2 indicated that the changes in salinity levels affected seed yield (g $Plant^{-1}$) of *Nigella sativa* L. Thus the seed yield in general decreased under the various saline treatments, especially at 6.3 dS m^{-1} treatment. Greatest seed yield was obtained in the 0.4 dS m^{-1} with the value of 5.1 g $Plant^{-1}$. The ANOVA indicated that the decrease in seed yield was highly significant in salinity treatments.

Table 2: Effect of salinity on seed yield and fixed oil

			Fixed oil content			
	Seed yield (g $plant^{-1}$)		Percentage		g $plant^{-1}$	
Salinity treatments (dS m^{-1})	Mean	SD	Mean	SD	Mean	SD
0.4	5.1	0.1	23.7	0.7	1.2	0.2
1.6	4.4	0.4	22.7	0.7	1.1	0.1
3.1	3.8	0.4	21.5	0.3	0.8	0.1
4.6	3.5	0.5	19.8	0.8	0.7	0.2
6.3	1.1	0.1	14.9	0.9	0.2	0.1
F Ratio	58.4***		67.1***		21.1***	

*p≤0.05 according to F-values (ANOVA-1), **p<0.01 according to F-values (ANOVA-1), ***p<0.001 according to F-values (ANOVA-1)

Table 3: Effect of salinity on fatty acids

		Salinity treatments (dS m^{-1})										
		0.4		1.6		3.1		4.6		6.3		
Fatty acids	Rt[a] (min)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F ratio
Saturated fatty acids												
Caprylic ($C_{8:0}$)	5.4	0.4	0.1	0.7	0.1	0.1	0.1	0.4	0.1	0.4	0.1	3.1**
Capric ($C_{10:0}$)	8.55	0.2	0.1	0.4	0.1	0.6	0.2	0.2	0.1	0.3	0.1	5.3***
Lauric ($C_{12:0}$)	10.4	0.4	0.1	0.6	0.2	0.6	0.2	0.3	0.2	0.3	0.2	3.1*
Myristic ($C_{14:0}$)	13.6	0.4	0.1	0.5	0.1	04	0.1	0.5	0.1	0.4	0.1	3.1*
Palmitic ($C_{16:0}$)	16.3	0.2	0.1	0.3	0.1	0.5	0.1	0.3	0.1	0.1	0.0	0.9
Stearic ($C_{18:0}$)	17.7	11.9	0.9	11.8	0.8	13.7	0.7	11.1	0.1	11.3	0.3	7.8***
Arachidic ($C_{20:0}$)	26.0	2.5	0.5	2.1	0.1	1.9	0.5	2.1	0.1	1.9	0.5	1.2
Total saturated fatty acids		16.0	0.4	16.4	0.7	21.4	0.2	14.9	0.2	14.7	0.3	121.5***
Unsaturated fatty acids												
Oleic ($C_{18:1}$)	20.3	24.8	0.8	24.5	0.5	18.2	5.7	23.5	0.5	23.1	0.1	1.3
Linoleic ($C_{18:2}$)	21.3	57.4	0.4	57.1	0.1	58.2	0.2	55.7	0.7	55.4	0.4	24.3***
Linolenic ($C_{18:3}$)	23.6	1.7	0.7	1.1	0.7	0.9	0.9	5.7	0.7	5.1	0.1	0.6
Total unsaturated fatty acids		83.9	0.9	82.7	0.2	77.3	0.3	84.9	0.9	83.6	0.6	29.5***
Total fatty acids		99.9		99.1		98.7		99.8		98.3		

Rt[a]: Retention time, *p≤0.05 according to F-values (ANOVA-1), **p<0.01 according to F-values (ANOVA-1), ***p<0.001 according to F-values (ANOVA-1)

Table 4: Effect of salinity on NPK contents and its uptake

	NPK											
							Uptake (mg plant $^{-1}$)					
	N (%)		P (%)		K (%)		N		P		K	
Salinity treatments (dS m^{-1})	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.4	3.1	0.1	0.4	0.1	0.9	0.2	15.9	0.9	2.0	1.0	4.6	0.6
1.6	2.9	0.4	0.3	0.2	0.7	0.2	12.8	0.8	1.3	0.3	3.1	0.1
3.1	2.7	0.4	0.2	0.1	0.5	0.1	10.3	0.3	0.8	0.1	1.9	0.1
4.6	2.4	0.4	0.1	0.0	0.3	0.1	8.4	0.3	0.4	0.1	1.1	0.1
6.3	1.3	0.3	0.1	0.0	0.2	0.1	1.4	0.4	0.1	0.0	0.2	0.3
F ratio	13.3***		4.3***		9.3***		1.0		7.7***		111.3***	

*p≤0.05 according to F-values (ANOVA-1), **p<0.01 according to F-values (ANOVA-1), ***p<0.001 according to F-values (ANOVA-1)

Effect of salinity on fixed oil content: As shown in Table 2, fixed oil content [percentage and yield (g plant^{-1})] decreased at all salinity treatments. The highest accumulation of fixed oil content was recorded at the lowest salinity level (control) with the values of 23.7% and 0.7 g plant^{-1}. ANOVA indicated that the changes in fixed oil were highly significant for salinity treatments.

Effect of salinity on fatty acids content: The gas chromatography analysis revealed the presence of ten different fatty acids identified (Table 3). In this study, Linoleic, Oleic and Stearic were detected as the major fatty acids of the fixed oil in all the treatments which changed under different salinity levels (Table 3). Fatty acids were identified in fixed oil isolated from *Nigella sativa* L. seeds belong to two chemical main classes. Unsaturated fatty acids was the major one, the remaining fraction saturated fatty acids formed the minor classes (Table 3). The highest amount of Linolenic (58.2%), Stearic (13.7) and total saturated fatty acids (21.4%) resulted from the treatments of 3.1 dS m^{-1} compared with other treatments. On the other hand the highest value of Oleic acid produced from the control treatment with the value 24.8%. The highest value (84.9%) of total unsaturated fatty acids resulted from the treatment of 4.6 dS m^{-1}. The ANOVA indicated that the changes in Caprylic, Capric, Stearic, Linoleic, total saturated fatty acids and total unsaturated fatty acids were highly significant for salinity treatments. The changes in Lauric and Myristic were significant. The changes in Palmitic, Arachidic, Oleic and Linolenic were insignificant (Table 3).

Effect of salinity on NPK content and uptake: Increase in salinity level caused a decrease in measured nutrient content such as macro elements (N, P and K) (Table 4). Salinity at 6.3 dS m^{-1} resulted in the lowest nutrient accumulations (1.3, 0.1 and 0.2%) and uptakes (1.4, 0.1 and 0.2 mg plant^{-1}) while the highest mineral contents (3.1, 0.4 and 0.9%) and uptakes (15.9, 2.0 and 4.6 mg plant^{-1}) were observed in the control treatment. ANOVA indicated that the changes in N, P and K as well as its uptakes were highly significant except N uptake was insignificant for salinity treatments.

DISCUSSION

The reduce of seed yield character under saline irrigation water treatments may be due to exposure to injurious levels of salinity causing a decrease of turgor which would result in a decrease of growth and development of cells, especially in stems and leaves (Kaufmann 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, 1973). When leaf size is smaller, the capacity to trap light decreases too and the capacity of total photosynthesis decreases, i.e. photosynthesis is restricted in salt stress conditions, with a subsequent reduction in plant growth, yield and performance (Hsiao, 1973). 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 agropeularia* (embrapa) plants (Slama *et al*., 2007; Cox and Jolhff, 1987; Viera *et al*., 1991). The effect of different salinity treatments on fixed oil and fatty acids may be due to its effect on enzyme activity and metabolism of fixed oil production (Burbott and Loomis 1969). 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).

Under Na^+ and Cl^- salts stresses, osmotic adjustment is usually achieved by the uptake of Na^+ and Cl^- from the soil solution. Balibrea *et al*. (2000) suggested that a great deal of harmless and compatible solutes were synthesized and accumulated in plant leaves, thus maintaining the osmotic balance. Osmotic adjustment by inorganic ions accumulation is less energy and carbon-demanding than adjustment by organic solutes (Yeo, 1983). Inorganic solutes formed the largest component contributing to osmotic adjustment in grapevines. The production of sufficient organic osmotica is metabolically expensive and potentially limits plant growth by consuming significant quantities of carbon that could otherwise be used for growth (Greenway and Munns, 1980). An alternative to producing organic osmotica is for the plants to accumulate a sufficiently high content of ions from the soil. The energetic cost of osmotic adjustment using inorganic ions is much lower than that of using organic molecules synthesized in the cells (Yeo, 1983; Hu and Schmidhalter, 1998). Thus by using this alternative mechanism of inorganic ion accumulation to adjust their osmotic potential, grapevines seem to save energy, which enables them to grow in less favorable conditions.

CONCLUSION

The *Nigella sativa* L. plants subjected to salinity treatments resulted in lower seed yield, fixed oil and nutrient content than untreated plants. Linoleic, Oleic and Stearic were detected as the major fatty acids of the fixed oil in all the treatments which changed under different salinity levels.

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