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# Changes in Lipids and Fatty Acids of *Nigella sativa* L. under Salinity Stress

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## Abstract

Total lipids and fatty acids of black seed/black cumin (*Nigella sativa* L.) have antimicrobial activities. Where as saline irrigation water (SIW) caused a dramatic effect on performance and physiological processes of agricultural crops. Selected tolerated plants to salinity stress is a focus of research and industry since salinity stress and total lipids (or fatty acids) yield are of major concern to increase total lipids production in arid and semi-arid regions. So, in this study, the lipids and fatty acids constituents of black cumin were determined under salinity stress factor *Nigella sativa* L. plants were subjected to different doses (0.25, 1, 2, and 3 g $l^{-1}$) of SIW. Evaluation of total lipids and fatty acids of *N. sativa* seeds were reported during two successive seasons. The highest amounts of total lipids (%) were recorded under the dose of 2 g $l^{-1}$ with the values of 30.4 and 28.6% during both seasons. The Maximum yields of total lipids were obtained in the $1gl^{-1}$ treatment with the values of 3.4 and 3.3 g Plant $^{-1}$. The greatest values (55.3, 22.1 and 55.3, 19.9) of the main fatty acids (linoleic and oleic) were recorded with 3 g $l^{-1}$ in both the seasons. The high accumulation in *N. sativa* lipid and fatty acids composition under salinity stress levels may be due to its effect of salinity on enzyme activity and metabolism of lipid production

## 1. Introduction

Black seed/black cumin (*Nigella sativa* L.) belongs to family *Ranunculaceae*, it is a various used medicinal herb [1]. Fatty acids of linoleic (>50%), oleic (>20%), palmitic (>10%) and stearic (<1%) were identified in lipids extracted from Egyptian variety of black cumin [2]. *Nigella sativa* L. fatty acids have an antimicrobial role [1].
Among different a biotic stress, saline irrigation water (SIW) becomes a critical factor. SIW caused a dramatic effect on performance and physiological processes of agricultural crops [3]**.** Salinity reduces the seed germination, resulting in inhibiting plant growth characters and yield [4, 5]. Hazards effect of plant growth characters and yield associated with high osmotic potential of the soil solution, imbalances of different nutrients and toxicity of ions [6]. Under high stress salt doses, different changes in total lipids and fatty acids composition were reported [7]. Salinity stress caused significant reduction total lipids extracted from black cumin seeds [8]. Fatty acids of polar lipids were changed with sodium chloride levels [9]. A response to salinity stress increases the content of sterol ester, free fatty acids and oleic acid, and decreases that of triacylglycerol and linoleic acid (C18H32O2) were recorded [10, 11]. Allakhverdiev [12] reported that the unsaturation of fatty acids in membrane lipids of *Synechocystis* sp. is associated with the ability of the photosynthetic machinery to tolerate salt stress. Total lipids of *B. hooglandii* increased with sodium chloride, but no changes in fatty acid composition were found [13]. It showed that an improvement of linoleic / linolenic acids ratio with increasing SIW levels [14]. Salinity stress reduced the total fatty acids content of canola plants. Polyunsaturated fatty acids decreased, while the monounsaturated ones increased as SIW increase [15]. Sodium chloride stress caused a decrease of fatty acids such as $C_{14:0}$, $C_{16:0}$ and $C_{16:1}$ while it produced an increase of polyunsaturated fatty acids such as $C_{18:4}$, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$ [16]. Variation effects were found in safflower fatty acids under salt stress

factor [17]. In this study, the total lipids and fatty acids constituents of black cumin were determined under salinity stress factor.

## 2. Experimental details

*2.1. Plan, site and methodology*

Experiments were carried out in a greenhouse at National Research Centre (NRC), Cairo, Egypt, in 2013 / 2014 and 2014 / 2015 seasons. Medicinal black cumin seeds were obtained from the Institute of Medicinal and Aromatic Plants (IMAP) located in Kalubia Governorate, Egypt. Uniform seeds were sown in plastic pots (30 cm diameter and 50 cm height) during the first week of November of both seasons; the pots were transferred in greenhouse adjusted to 24/18°C, 90/60% RH day/night and light intensity ~3700 µmol.$m^{-2}$.$s^{-1}$. Each pot was filled with 10 kg of air-dried soil (sandy soil). Three weeks after sowing, the seedlings were thinned to three plants per pot. 45 days from sowing date plants were divided into five groups were subjected to different levels of saline irrigation water (SIW), 0.25 (tap water as control), 1, 2, and 3 g $l^{-1}$. To prepare irrigation water with different salinity levels, highly soluble NaCl salt were used. The salt was used due to its presence in water naturally [18]. Plants subjected to saline irrigation water every 7 days however all pots were leached by tap water every 28 days (If there was no leaching when irrigating with saline water, it may induce more salt build up in pots). All Cultural practices were presented according to recommendations of Ministry of Agriculture, Egypt. Mechanical and chemical properties of the soil used in this experiment were done according to Jackson [19, 20] and are presented in **Table1**.

**Table 1:** Physical and chemical properties of the soil used

| Clay | Silt | Sand | OM | N | P | K | pH | EC ($dsm^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| (%) | | | | | | | | |
| 38.0 | 36.0 | 26.0 | 1.3 | 0.3 | 0.1 | 0.1 | 7.7 | 0.6 |
| Note: OM= organic matter, EC= electronic conductivity. | | | | | | | | |

*2.2. Harvesting*

At fruiting stage, plants were harvested. Seed yields (g $Plant^{-1}$) were recorded.

*2.3. Extraction of total lipds*

The seeds (ten grams) 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 total lipids [21]. In addition, total total lipids yield (% and g $Plants^{-1}$) were calculated by using the dry seeds of both seasons. The total lipids extracted from black cumin seeds were collected in both seasons from each treatment to identify the fatty acids.

*2.4. Gas chromatography*

The fatty acid content of the total lipids was investigated by GC analysis of their methyl esters. A total lipid (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, was hed with water, and 1 ml used for GC analysis [22].

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

*2.5. Gas chromatography mass spectrometry (GC- MS)*

GC- MS analyses of the oils were carriedout on HP GC- MS 6890-5 973 model instruments. The GC column used SP23 80 capillary column 60m X 0.25mm X 0.20 µm. The 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.

*2.6. 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) . Quantified ion of fatty acid methyl esters was obtained directly from GC peak area using Chemstation 8.02 software and expressed as percent ages.

*2.7. Statistical analysis*

For experiments, one factor was considered: salinity levels. For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual were potted. The experimental design followed a complete random block design. According to Snedecor [23], the averages of data were statistically analysed 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 [24].

## 3. Results and discussion

*3.1. Effect of salinity on total lipids content and fatty acids constituents*

Total lipids contents (%) and yield (g Plant $^{-1}$) were affected by different salinity doses during both seasons **(Table 2)**. Generally the different doses of salinity caused an increase in total lipids percentages compared with control. The highest amounts of total lipids (%) were recorded under the dose of 2 g $l^{-1}$ with the values of 30.4 and 28.6% during the first and second seasons respectively. The lowest total lipids percentages were obtained at 0.25 g $l^{-1}$ with the values of 15.8 and 13.5%. The doses of 1 and 2 g $l^{-1}$ resulted in an increment in total lipids yield while it decreases fewer than 3 g $l^{-1}$. Greatest yield of total lipids were obtained in the 1 g $l^{-1}$ treatment with the values of 3.4 and 3.3 g Plant $^{-1}$ during the first and second seasons respectively but the lowest were obtained from 3 g $l^{-1}$ which recorded 2 and 1.7 g Plant $^{-1}$. Analysis of variance (ANOVA) indicated that the changes in lipids contents (%) and yield were highly significant but insignificant for yield at first season.

**Table 2.** Effect of salinity on total lipids.

| SIW treatments (g $l^{-1}$) | Total lipids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (%) | | | | Yield (g Plant $^{-1}$) | | | |
| | Seasons | | | | | | | |
| | 1st | | 2nd | | 1st | | 2nd | |
| | M | SD | M | SD | M | SD | M | SD |
| 0.25 (cont.) | 15.8 | ± 3.0 | 13.5 | ± 0.5 | 2.7 | ± 0.3 | 2.3 | ± 0.2 |
| 1 | 22.5 | ± 0.5 | 21.8 | ± 0.2 | 3.4 | ± 0.4 | 3.3 | ± 0.3 |
| 2 | 30.4 | ± 0.4 | 28.6 | ± 0.6 | 3.0 | ± 0.1 | 2.9 | ± 0.1 |
| 3 | 27.9 | ± 0.1 | 24.8 | ± 0.8 | 2.0 | ± 0.1 | 1.7 | ± 0.3 |
| F ratio | 53.3*** | | 383.2*** | | 1.8 | | 25.6*** | - |
| Note: SIW= saline irrigation water; M= mean; SD = standard deviations ; cont. = control | | | | | | | | |

GC/MS and GC analysis revealed the presence of nine different fatty acids identified under salinity treatments of both seasons **(Tables 3 and 4)**. Isolated fatty acids belong to two chemical classes. Unsaturated fatty acids was the major one, the remaining fractions as saturated fatty acids formed the minor classes. Unsaturated fatty acids were oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) while saturated fatty acids were caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), stearic (C18:0) and arachidic (C20:0). The maim fatty acids were linoleic and oleic (more than 65%). Total saturated fatty acids (TSFA) were gradually decreased as salinity level increase while total unsaturated fatty acids (USFA) were increased. The highest amounts of TSFA were recorded with 0.25 (control) treatments with the values of 24.2 and 28.2% while the highest amounts (80.1 and 77%) of USFA were obtained under the treatment of 3 g $l^{-1}$. The main fatty acids (linoleic and oleic) were gradually increased as salinity level increase. The highest values (55.3, 22.1 and 55.3, 19.9) of main fatty acids were recorded with 3 g $l^{-1}$.

During the first season ANOVA indicated that the changes in lauric, myristic, stearic, arachidic, TSFA, linoleic, linolenic and USFA were highly significant; caprylic and linoleic were significant; capric was insignificant.
At the second season the changes in, capric, lauric, myristic, oleic and linoleic were highly significant; caprylic, arachidic, TSFA and USFA were moderate significant; stearic and linolenic were significant. Seminar fatty acids were found by Ahmad [1] in black cumin seeds.
Exposure to the high salinity level (3 g $L^{-1}$) which produces a reduction in turgor and decrease in cells growth and development. Cell growth is the most important process affected by salinity stress, which affect in plant size [25]. The capacity to trap light and the capacity of the total photosynthesis depending on the leaf size, photosynthesis is restricted in salinity stress factor with a subsequent reduction in seed yield and lipid content [25]. It is well known that a plant cell membrane undergoes a number of alterations in its lipid and fatty acid composition in response to changes in environmental factors. For example, in sorghum and tobacco plants, exposure environmental induces various changes in the level of unsaturated fatty acids [26, 27].
Some previous results in agreement with our results, changes in fatty acids were observed in *Coriandrum sativum* [28]. Harrathi [29], indicating that the response to salt constraint depends on plant species. Different changes in fatty acids were found under salinity stress doses [9]. It found that various changes in linoleic and linolenic acids under soil salinity levels [14]. Salinity stress reduced the some fatty acids content of canola plants [15]. Salinity stress caused significant reduction in total lipids extracted from black cumin seeds [8].

**Table 3.** Effect of salinity on fatty acids constituents during first season.

| Fatty acids | RT | 1st season | | | | | | | | F ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | | SIW treatments (g $l^{-1}$) | | | | | | | | |
| | | 0.25 (cont.) | | 1 | | 2 | | 3 | | |
| | | M | SD | M | SD | M | SD | M | SD | |
| Saturated fatty acids | | | | | | | | | | |
| Caprylic (C8:0) | 6.7 | 0.8 | ± 0.2 | 0.6 | ± 0.1 | 0.4 | ± 0.1 | 0.3 | ± 0.1 | 8.4** |
| Capric (C10:0) | 12.7 | 2.6 | ± 0.1 | 2.1 | ± 0.1 | 2.0 | ± 0.1 | 1.9 | ± 0.1 | 1.1 |
| Lauric (C12:0) | 17.9 | 3.8 | ± 0.2 | 3.7 | ± 0.3 | 1.5 | ± 0.5 | 1.4 | ± 0.1 | 54.3*** |
| Myristic (C14:0) | 22.3 | 8.7 | ± 0.3 | 8.9 | ± 0.1 | 10.6 | ± 0.4 | 10.9 | ± 0.1 | 57.2*** |
| Stearic (C18:0) | 24.8 | 7.5 | ± 0.5 | 7.1 | ± 0.1 | 6.4 | ± 0.4 | 1.7 | ± 0.3 | 170.1*** |
| Arachidic (C20:0) | 26.5 | 0.8 | ± 0.2 | 0.7 | ± 0.3 | 0.9 | ± 0.1 | 3.1 | ± 0.1 | 106.3*** |
| Total saturated fatty acids | | 24.2 | ± 0.1 | 23.1 | ± 0.1 | 21.8 | ± 0.2 | 19.3 | ± 0.3 | 296.4*** |
| Unsaturated fatty acids | | | | | | | | | | |
| Oleic (C18:1) | 29.4 | 18.9 | ± 0.1 | 21.6 | ± 0.4 | 21.7 | ± 0.3 | 22.1 | ± 0.1 | 95.5*** |
| Linoleic (C18:2) | 32.7 | 51.8 | ± 0.2 | 51.9 | ± 0.1 | 52.8 | ± 0.2 | 55.3 | ± 0.3 | 177.1*** |
| Linolenic (C18:3) | 35.9 | 3.2 | ± 0.2 | 3.1 | ± 0.1 | 2.9 | ± 0.1 | 2.7 | ± 0.3 | 3.9* |
| Total unsaturated fatty acids | | 73.9 | ± 0.1 | 76.6 | ± 0.4 | 77.4 | ± 0.4 | 80.1 | ± 0.1 | 229.8*** |
| Total fatty acids | | 98.1 | | 99.7 | | 99.2 | | 99.4 | - | - |
| Note: SIW= saline irrigation water; M = mean; SD = standard deviations; RT = retention time; ; cont.= control | | | | | | | | | | |

Generally, environmental conditions resulted in significant changes on the chemical content of *N. sativa*. NPK x foliar nutrition led to higher biochemical contents of *N. sativa* than untreated plants [30]. Salinity stress caused significant changes in oil composition isolated from *N. sativa* seeds [31, 32]. Foliar nutrition and ammonium sulphate caused a highly significant increase in *N. sativa* lipids [33].
Under salinity conditions, osmotic adjustment is usually achieved by the uptake of $Na^+$ and $Cl^-$ from the soil solution. Balibrea [24] 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 [35]. 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 [36]. 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 [35, 37]. 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 favourable conditions.

**Table 4.** Effect of salinity on fatty acids constituents during second season.

| Fatty acids | RT | 2nd season | | | | | | | | F ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | | SIW treatments (g $l^{-1}$) | | | | | | | | |
| | | 0.25 (cont.) | | 1 | | 2 | | 3 | | |
| | | M | SD | M | SD | M | SD | M | SD | |
| Saturated fatty acids | | | | | | | | | | |
| Caprylic (C8:0) | 6.7 | 0.9 | ± 0.1 | 0.8 | ± 0.2 | 0.6 | ± 0.1 | 0.4 | ± 0.1 | 8.4** |
| Capric (C10:0) | 12.7 | 5.7 | ± 0.3 | 2.5 | ± 0.5 | 2.2 | ± 0.2 | 1.8 | ± 0.2 | 91.5*** |
| Lauric (C12:0) | 17.9 | 5.5 | ± 0.5 | 5.7 | ± 0.3 | 5.1 | ± 0.1 | 2.8 | ± 0.2 | 55.2*** |
| Myristic (C14:0) | 22.3 | 7.8 | ± 0.2 | 7.9 | ± 0.1 | 8.4 | ± 0.4 | 8.9 | ± 0.1 | 14.0*** |
| Stearic (C18:0) | 24.8 | 6.9 | ± 0.1 | 6.8 | ± 0.2 | 6.4 | ± 0.4 | 6.1 | ± 0.2 | 7.4* |
| Arachidic (C20:0) | 26.5 | 3.1 | ± 0.1 | 3.8 | ± 0.2 | 3.6 | ± 0.4 | 2.5 | ± 0.5 | 8.7** |
| Total saturated fatty acids | | 29.2 | ± 0.2 | 27.5 | ± 0.5 | 26.3 | ± 0.3 | 22.5 | ± 0.5 | 93.7*** |
| Unsaturated fatty acids | | | | | | | | | | |
| Oleic (C18:1) | 29.4 | 17.5 | ± 0.5 | 17.9 | ± 0.1 | 18.6 | ± 0.4 | 19.9 | ± 0.1 | 31.0*** |
| Linoleic (C18:2) | 32.7 | 49.5 | ± 0.5 | 51.4 | ± 0.4 | 53.2 | ± 0.2 | 55.3 | ± 0.3 | 136.7*** |
| Linolenic (C18:3) | 35.9 | 2.4 | ± 0.4 | 2.5 | ± 0.5 | 1.6 | ± 0.4 | 1.8 | ± 0.2 | 3.8* |
| Total unsaturated fatty acids | | 69.4 | ± 0.4 | 71.8 | ± 0.2 | 73.4 | ± 0.4 | 77.0 | ± 0.3 | 13.0** |
| Total fatty acids | | 98.6 | | 99.3 | | 99.6 | | 99.5 | - | - |

Note: SIW= saline irrigation water; M = mean; SD = standard deviations; RT = retention time; ; cont. = control

The high accumulation in *N. sativa* lipid composition under salinity stress levels may be due to its effect of salinity on enzyme activity and metabolism of lipid production [38]. Different changes other than the changes in lipid or fatty acid can be happened under salinity stress. The high accumulation in proline and soluble sugars with salinity stress levels may be due to proline is regarded as a source of energy, carbon, and nitrogen for recovering tissues under salt stress conditions [39]; salinity levels enhanced the plant to preserve sugars for sustained metabolism, prolonged energy supply, and for better recovery after stress relief [40-42, 8].
On the other hand some new studies indicated that salinity treatment reduced plant growth characters and increased the menthone and pulegone contents of mint herb [43]. The positive or negative effects of salt stress on basil depend on the degree of tolerance of the different genotypes [44]. The accumulative effect of increasing salinity reduced stem height and elongation [45].

## Conclusions

Total lipids (percentages) were increased under salinity stress conditions. The highest yields (g $Plant^{-1}$) of total lipids were recorded in the 1 g $l^{-1}$ treatment. The maim fatty acids identified under salinity stress were linoleic and oleic. The highest values of main fatty acids were recorded with 3 g $l^{-1}$. The changes in lipids contents (%) and yield were highly significant but insignificant for yield at first season. During the first season the changes in lauric, myristic, stearic, arachidic, TSFA, linoleic, linolenic and USFA were highly significant; caprylic and linoleic were significant; caprylic was insignificant. At the second season the changes in, capric, lauric, myristic, oleic and linoleic were highly significant; caprylic, arachidic, TSFA and USFA were moderate significant; stearic and linolenic were significant.

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