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# Salt effects on shoot growth and essential oil yield and composition in safflower (*Carthamus tinctorius* L.)

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Salinity effects on growth and essential oil composition of Tunisian safflower (*Carthamus tinctorius*) shoots grown in hydroponic medium were investigated. Plants treated with 25 mM NaCl showed remarkable morphological modifications and decrease in shoot dry matter. Under 50 and 75 mM NaCl, plants showed a withering and a drastic reduction in dry matter (50% and 70%, respectively). Under NaCl 25 mM, essential oil yield increased significantly ($p<0.05$) up to 70% and 27% respectively in plants from Tazarka and Kairouan provenances compared with the control. The major compound in Tazarka essential oil was found to be 1,8-cineole (23.5%) followed by methyl eugenol (18.0%), 1-pentadecene (9.1%) and camphene (9.0%). In plants from Kairouan provenance, the major compounds were 1-pentadecene (22.9%), methyl-eugenol (11.8%), linalool (8.1%) and camphene (7.9%). Whatever the origin, the application of 25 mM resulted in remarkable changes in the content and the percentage of the main oil components with the effect being more pronounced in plants from Tazarka.

**Keywords:** *Carthamus tinctorius*; essential oil composition; salinity; shoots; 1,8-cineole; 1-pentadecene

## 1. Introduction

Salinity is one of the main environmental constraints limiting plant growth and productivity (1). In general, salinity reduces plant growth and leads to physiological and biochemical changes, like loss of chloroplast activity, decreased photosynthetic rate, increased photorespiration rate, disturbance of structure and functions of cell membranes, and changes in primary and secondary metabolisms, among other (2). In addition, increased concentration of sodium and chloride disturbs the plant–water relations due to decreased availability of water from soil solution as a result of lowered osmotic potential (1). The underlying causes of salinity injury include changes in allocation patterns such as an increased allocation to root at the expense of leaf growth, partial stomatal closure and premature leaf senescence, which affect the photosynthetic machinery and the metabolic processes (3). To cope with the salt stress, plants have evolved a plethora of biochemical mechanisms including amino acid and amine accumulation, modification of lipid composition of the plasma membrane, increase in phenolic production and in some cases increase in essential oil (EO) synthesis (4). Regarding the EO production under salt stress, literature data are scant and contradictory. At this point, Ansari et al. (5) reported that the EO yield decreased with the increased water salinity in three *Cymbopogon* species. In contrast, Ben Taarit et al. (6) and Neffati and Marzouk (7) found that moderate salinity resulted in increased EO content in *Salvia officinalis* and *Coriandrum sativum*, respectively.

*Carthamus tinctorus* (Asteraceae), commonly known as safflower, is used in medicinal, pharmaceutical and food coloring fields. In the last years, this species has received special attention due to its high seed oil content and quality, and other photochemicals such as colorants, minerals, phenolics and EOs (8). This species is considered a moderately tolerant plant to salinity (9) and the germinative stage was reported to be more sensitive to salt stress than the vegetative one (10). In Tunisia, this plant is cultivated for its valuable seeds and is distributed in the arid and semiarid areas of the country. Despite *C. tinctorius* representing a promising source of a wide array of bioactive components, physiological and biochemical responses of this spice to salinity are poorly known and more information is needed. Such data are important for developing suitable agronomic strategies for crop production in saline soils. The aim of this work was to evaluate the effects of NaCl on growth, EO yield and composition of safflower shoots.

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## 2. Experimental

### 2.1. Plant material and culture

Safflower (*Carthamus tinctorius*) akenes were collected from cultivated plants from two provenances: Tazarka (North-east of Tunisia) and Kairouan (Center of Tunisia) on July 2007. Seeds were removed and germinated at 25°C. Fifteen-day-old seedlings were grown in quarter-strength Hoagland's (1940) solution added with 0, 25, 50 and 75 mM NaCl. The culture was placed in a greenhouse at 22°C day maximum and 18°C night minimum, under artificial light of 150 μmol/$m^2$/s (6000 lux) with 16-hour photoperiod and 64–86% relative air humidity. Nutrient solution was continuously aerated.

The final harvest occurred after two weeks. For the determination of the dry weight (DW), shoots were oven-dried at 60°C for 48 hours. Sodium ($Na^+$) and potassium ($K^+$) were determined by flame emission spectrophotometry (Jenway PFP7) after nitric acid extraction ($HNO_3$ 0.5%) from a fine powder of dry matter.

### 2.2. Essential oil extraction

Three lots of 50-g dry shoots from each provenance were hydrodistilled for 90 minutes. Hydrodistillation was performed by a simple laboratory Quick-fit apparatus, which consisted of a 1000-mL steam generator flask, a distillation flask, a condenser and a receiving vessel. EOs were collected from the distillate in diethyl ether using a liquid–liquid isolation. To quantify EOs and their main constituents, the 6-methyl-5-hepten-2-one was used as internal standard. EO yield was then estimated on the basis of plant dry matter weight.

### 2.3. Gas chromatography analysis

Gas chromatography (GC) analyses were performed using a Hewlett–Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP Innowax (polyethylene glycol) column (30 m×0.25 mm, 0.25 μm film thickness) and an apolar HP-5 (5%-phenyl-methylpolysiloxane) column were used. The carrier gas was $N_2$ (U) with a flow rate of 1.6 mL/minute. The split ratio was 60:1. The oven temperature was initially held at 35°C for 10 minutes, then raised to 205°C at a rate of 3°C/minute and finally held at 225°C for 10 minutes. Injector and detector temperatures were held, respectively, at 250° and 300°C. Quantitative data were obtained from the electronic integration of the FID peak areas.

### 2.4. Gas chromatography–mass spectrometry

GC–mass spectrometry (GC–MS) analyses were performed on a gas chromatograph HP 5890 (II) coupled to a HP 5972 mass spectrometer with electron impact ionization (70 eV). An HP-5MS capillary column (30 m×0.25 mm, 0.25 μm film thickness) was used. The column temperature was programmed to rise from 50° to 240°C at a rate of 5°C/minute. The carrier gas was helium with a flow rate of 1.2 mL/minute; split ratio was 60:1. Scan time and mass range were 1 s and 40–300 *m*/*z*, respectively.

### 2.5. Compound identification

EO components were identified by comparison of their retention indices (RI) relative to ($C_8$–$C_{22}$) *n*-alkanes with those of authentic compounds analyzed under the same conditions (11). Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library and other published mass spectra (12).

### 2.6. Statistical analysis

Statistical analysis was performed with Statistica™ software, using two-way analysis of variance (ANOVA) and Newman–Keuls test for *post hoc* mean comparison at the significance level of 0.05.

## 3. Results and discussion

### 3.1. Effect of salt on plant morphology, growth and ion accumulation

The presence of 25 mM NaCl in the culture medium resulted in morphological changes. In both provenances, leaf yellowing, length reduction of roots and thickness of stems were observed. After two months of treatment, salt-treated plants showed significantly ($p$<0.05) lower shoot dry weight when compared with the control plants. Plants from Kairouan were more affected by salt treatment than those from Tazarka and the decrease in shoot dry weight reached 28% and 15%, respectively (Figure 1). At 50 and 75 mM of NaCl, these symptoms became more pronounced, reaching 50–70% of reduction in plants from Kairouan (Figure 1). For leaves, the decrease in dry weight under salinity (data not shown) concerned mainly the leaf expansion (total leaf area: 50% of control in plants from Kairouan for 25 mM NaCl). In contrast to leaf area, leaf number appears to be less affected especially in plants from Tazarka (Figure 2). Bassil and Kaffka (13) found that safflower response to increasing salinity included reduced plant height, biomass, leaf area, capitula number and order, and earlier maturation. The two provenances studied in the present work showed different shoot dry weights. The higher values observed in plants from Kairouan were due to a higher shoot growth rate that could be genetically controlled. In addition, Kairouan was shown to be more sensitive to salt stress than Tazarka in terms of shoot biomass production. Ashraf and Orooj (14) reported that

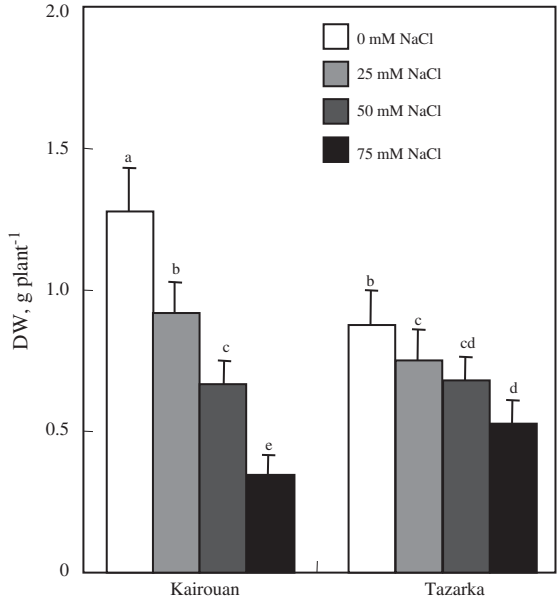


Figure 1. Salinity effect on shoot dry matter of two Tunisian *Carthamus tinctorius* provenances (Kairouan and Tazarka). Fifteen-day-old plants were grown for two weeks in the presence of 0, 25, 50 or 75 mM NaCl. Means of eight replicates±confidence intervals at $p$=0.05. Means sharing a same letter are not significantly different at $p$=0.01 (analysis of variance and mean comparison with Newman–Keuls test).

increased NaCl levels in the growth medium caused a marked reduction in *ajwain* (*Trachyspermum ammi*) vegetative growth. Neffati and Marzouk (15) showed that salt treatments significantly reduced the biomass of stems and leaves of coriander plants (*C. sativum*). Salt stress also reduced total leaf area of *C. tinctorius*. Attia et al. (16) signaled that salt restricted whole plant biomass deposition rate of basil (*Ocimum basilicum*) by diminishing both leaf number and leaf expansion. These data are partially in accordance with our findings and suggest that growth responses to salt stress depend on species/cultivars.

Results depicted in Figure 3 showed that the concentration of $Na^+$ in the shoots increased with the increasing salinity, while the concentration of $K^+$ showed reciprocal trends. Also, the range of variation in the concentration of these ions was not significant in plants from both provenances. These results are in good agreement with previous studies that reported increased accumulation of $Na^+$ and decreased concentration of $K^+$ in salt-treated plants (17). They attributed the decrease of the concentration of $K^+$ to the decrease of its uptake and transport to shoots. Similarly, Attia et al. (18) showed that long-term mild salinity resulted in mineral nutrient shortage (including $K^+$) and this salt-induced default in mineral nutrition was the major cause of growth restriction in *Arabidopsis thaliana*.

In light of these physiological results, it was not possible to use altered and withered samples for the related experiments. So, the following results concern only NaCl 25 mM treatment and the control (NaCl 0 mM).

### 3.2. *Essential oil yield, composition and contents*

Figure 4 shows the contents of EO in salt-treated and control plants. As can be seen, the EO content was significantly ($p$<0.05) higher in plants from Kairouan. The average mean values (20.8 µg/g DW) in these plants were approximately five-fold higher than those from Tazarka (4.5 µg/g DW). Interestingly, the application of 25 mM NaCl resulted in significant increase in EO content in plants from both provenances. A salt-induced increase in EO yield

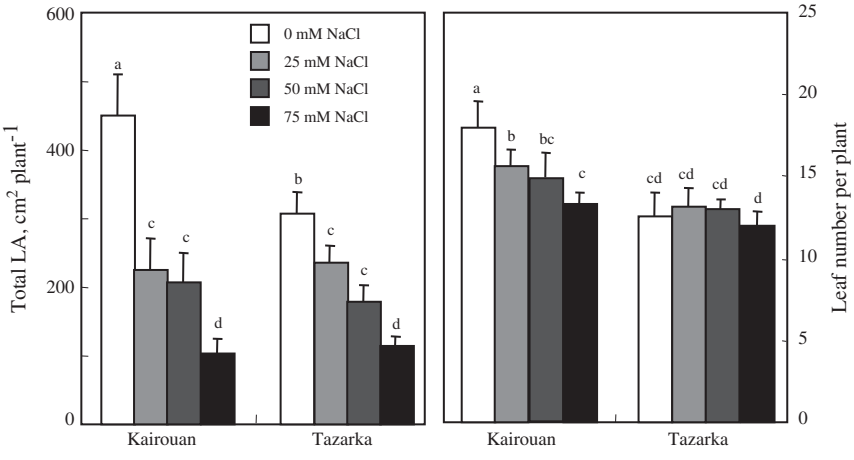


Figure 2. Salinity effect on total leaf area and number of two Tunisian *Carthamus tinctorius* provenances (Kairouan and Tazarka). Fifteen-day-old plants were grown for two weeks in the presence of 0, 25, 50 or 75 mM NaCl. Means of eight replicates±confidence intervals at $p$=0.05. Means sharing a same letter are not significantly different at $p$=0.01 (analysis of variance and mean comparison with Newman–Keuls test).

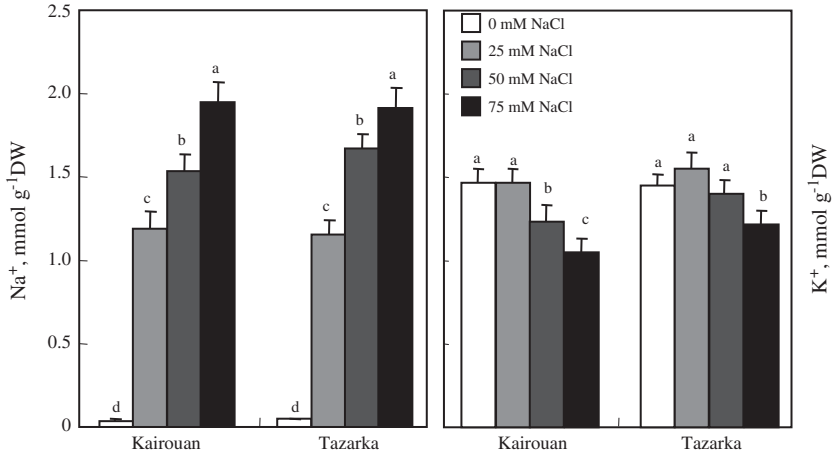


Figure 3. Salinity effect on $Na^+$ and $K^+$ concentrations in shoots of two Tunisian *Carthamus tinctorius* provenances (Kairouan and Tazarka). Fifteen-day-old plants were grown for two weeks in the presence of 0, 25, 50 or 75 mM NaCl. Means of eight replicates±confidence intervals at $p$=0.05. Means sharing a same letter are not significantly different at $p$=0.01 (analysis of variance and mean comparison with Newman–Keuls test).

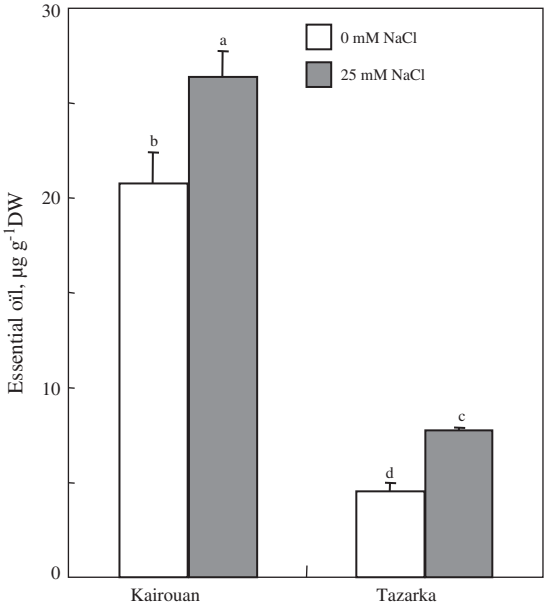


Figure 4. Salinity effect on shoot essential oil contents (µg/g DW) of two Tunisian *Carthamus tinctorius* provenances (Kairouan and Tazarka). Means of three replicates±confidence intervals at $p$=0.05. Means sharing a same letter are not significantly different at $p$=0.01 (analysis of variance and mean comparison with Newman–Keuls test).

was reported earlier in some other species such as *S. officinalis* (6, 19) and *C. sativum* (7). Such stimulation under a moderate salinity could be due to a higher oil gland density and an increase in the absolute number of glands produced prior to leaf emergence (20). In contrast, Ansari et al. (5) reported that essential oil content and yield decreased with the increasing water salinity in three *Cymbopogon* species.

Twenty-four components accounting for 90% of total EO were identified (Table 1). In plants from Kairouan, 1-pentadecene (22.9%), methyl-eugenol (11.9%), linalool (8.1%) and camphene (7.9%) were found as the main components. In plants from Tazarka, 1,8-cineole (23.5), methyl-eugenol (18.0%), 1-pentadecene (9.1%) and camphene (9.0%) were the major compounds. These results suggest the occurrence of two chemotypes: 1-pentadecene for Kairouan and 1,8-cineole for Tazarka.

Under NaCl 25mM, the percentages of the main compounds varied in the same way (Tables 1 and 2). In salt-treated plants, an increase in the proportion of 1-pentadecene was observed in plants from both provenances. Such NaCl concentration induced remarkable decrease in the proportion of methyl-eugenol in plants from both provenances. For the remaining compounds, an irregular variation in the proportion of the main components was observed. For example, the 1,8-cineole percentage increased in plants from Kairouan while it decreased in plants from Tazarka. The reciprocal trend was observed for camphene; its percentage increased in plants from Tazarka while it decreased in plants from Kairouan. At this point, it seems that salt stress induces modification of the metabolic pathways of the oil constituents.

In conclusion, increased NaCl concentration resulted in a significant decrease in the production of dry matter in *C. tinctoruis*. It also affected leaf area and number. The concentration of $Na^+$ increased significantly ($p$<0.05) in salt-treated plants, while that of $K^+$ decreased. Moderate salinity (25 mM NaCl) stimulates EO oil production in safflower shoots and induced chemotype modification in Tazarka EO that became

Table 1. NaCl effect on shoot essential oil composition (%) of two Tunisian *Carthamus tinctorius* provenances (Kairouan and Tazarka).

| | | | Kairouan | | Tazarka | |
|---|---|---|---|---|---|---|
| | | | NaCl, mM | | | |
| Component | $RI_{Cal}$ | $RI_{Lit}$ | 0 | 25 | 0 | 25 |
| α-Pinene | 1032 | 1032 | 1.02±0.9 | tr | tr | tr |
| α-Thujene | 1035 | 1035 | 4.02±1.5 | 1.30±0.5 | 2.57±0.6 | 0.97±1.5 |
| Camphene | 1076 | 1076 | 7.94±2.1 | 6.50±2.1 | 8.99±1.5 | 11.40±2.1 |
| β-Pinene | 1118 | 1118 | 0.48±0.2 | tr | tr | 0.11±0.2 |
| Sabinene | 1132 | 1132 | 2.02±0.9 | tr | 0.33±0.1 | tr |
| Myrcene | 1174 | 1174 | 0.13±0.1 | tr | tr | tr |
| α-Terpinene | 1188 | 1188 | 0.45±0.1 | 0.50±0.2 | tr | tr |
| Limonene | 1203 | 1203 | 0.45±0.1 | tr | tr | 0.11±0.1 |
| 1,8-Cineole | 1211 | 1213 | 4.68±1.1 | 9.70±3.4 | 23.53±5.1 | tr |
| *trans*-2-Hexenal | 1370 | 0.16±0.0 | tr | tr | 0.17±0.0 | |
| γ-Terpinene | 1255 | 1255 | 5.94±0.1 | tr | 0.27±0.1 | 0.24±0.1 |
| β-Ocimene | 1266 | 1265 | 0.20±0.1 | tr | tr | tr |
| *p*-Cymene | 1280 | 1280 | 0.93±0.4 | tr | tr | 0.16±0.4 |
| Terpinolene | 1290 | 1290 | 0.08±0.0 | tr | tr | tr |
| *Z*-3-Hexenol | 1370 | 1391 | 3.62±1.0 | 3.72±1.0 | 2.36±0.5 | 2.07±0.1 |
| Linalool | 1553 | 1553 | 8.11±2.6 | 2.31±0.6 | 5.20±1.2 | 0.53±2.6 |
| Bornyl acetate | 1597 | 1597 | 0.41±0.1 | 0.29±0.1 | 0.69±0.1 | 0.33±0.1 |
| Terpinene 4-ol | 1611 | 1611 | 1.78±0.5 | 3.02±0.5 | 1.30±0.1 | 1.86±0.5 |
| β-Caryophyllene | 1612 | 1612 | 2.68±1.1 | 0.44±0.2 | 1.74±0.5 | 0.16±1.1 |
| α-Terpineol | 1709 | 1706 | 5.08±2.0 | 4.17±2.2 | 7.39±2.3 | 0.54±2.0 |
| 1-Pentadecene | 1829 | 22.9±5.5 | 27.80±4.9 | 9.11±3.0 | 35.95±5.5 | |
| Methyl eugenol | 2030 | 2030 | 11.8±3.1 | 7.70±3.1 | 18.00±4.3 | 11.73±3.1 |
| Spathulenol | 2144 | 2146 | 0.17±0.1 | 0.36±0.1 | 0.44±0.9 | 0.71±0.1 |
| Cynnamyl acetate | 2155 | 1.88±0.6 | 1.58±0.6 | 3.51±1.1 | 0.99±0.6 | |
| Eugenol | 2186 | 2192 | 0.66±0.2 | 1.96±0.2 | 1.96±0.6 | 0.70±0.2 |

Notes: Fifteen-day-old plants were grown for two weeks in the absence (control) or in the presence of 25 mM NaCl. Means of three replicates±confidence intervals at $p$=0.05. Compounds are listed in order of their elution from HP-Innowax column. RIcal, retention indices on the HP-Innowax column were calculated using homologous series of *n*-alkanes ($C_8$–$C_{22}$); RIlit, retention indices on HP-Innowax reported in literature (21), tr: traces (<0.05%).

Table 2. NaCl effect on the shoot contents (μg/g dry weight) of the major essential oil components in two Tunisian *Carthamus tinctorius* provenances (Kairouan and Tazarka).

| | Kairouan | | Tazarka | |
|---|---|---|---|---|
| | 0 mM NaCl | 25 mM NaCl | 0 mM NaCl | 25 mM NaCl |
| Camphene | 1.59 | 2.87 | 0.29 | 0.89 |
| 1,8-Cineole | 0.92 | 2.58 | 0.81 | tr |
| 1-Pentadecene | 4.61 | 7.70 | 0.27 | 3.64 |
| Methyl eugenol | 2.55 | 1.83 | 1.12 | 0.94 |

Note: Fifteen-day-old plants were grown for two weeks in the absence (control) or in the presence of 25 mM NaCl.

1-pentadecene. The observed modifications might represent a set of adaptive mechanisms employed by *C. tinctorius* to cope with the deleterious effects of salt stress.

Interestingly, this study added new information about the physiological and biochemical responses of *C. tinctorius* to salt stress and may be useful for improving EO production.

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