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Effect of salinity on plant growth and biological activities of *Carthamus tinctorius* L. extracts at two flowering stages

Nidhal Salem · Kamel Msaada · Wissal Dhifi · Ferid Limam · Brahim Marzouk

Abstract In the present study, we were interested in the effect of salt stress on phenolic and carotenoid contents, antioxidant and antimicrobial activity of two varieties of *Carthamus tinctorius* (Jawhara and 104) flowers. For this purpose, *C. tinctorius* flowers from plants grown under four saline treatments (0, 5, 10 and 15 g/L NaCl) were collected at two development stages. As salinity increased up to 10 g/L, results showed that total phenols, flavonoids, condensed tannins and carotenoid contents increased with salinity. Such variability might be of great importance in terms of valorizing this plant as a source of naturally secondary metabolites. Furthermore, our results showed an enhancement of antioxidant activity which was evaluated by four different test systems (DPPH, β-carotene–linoleic acid, chelating and reducing power assays) with increasing stress severity. Obtained results showed that, for the two varieties, salt effect was more pronounced at post flowering stage than full flowering one. The sensitivity test of the methanolic extracts of the harvested flowers was applied against seven human pathogenic bacteria and three yeast strains. Salinity reduced significantly the antimicrobial activity of flower extracts.

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N. Salem · K. Msaada (✉) · F. Limam · B. Marzouk
Laboratory of Bioactive Substances, Biotechnology Center in Borj-Cedria Technopol, BP. 901, 2050 Hammam-Lif, Tunisia
e-mail: msaada_kamel@hotmail.com; msaada_kamel@yahoo.fr

W. Dhifi
UR Ecophysiologie Environnementale et Procédés Agroalimentaires, BiotechPole de Sidi Thabet Université de La Manouba, Manouba, Tunisia

Introduction

The production of medicinal and aromatic plants is sharply limited by abiotic constraints especially salinity (Ben Taârit et al. 2012). Plants subjected to high salinity levels undergo various physiological and biochemical changes leading to numerous modifications in the structure and function of cell membranes (Ben Taârit et al. 2010). Salinity stress is capable of triggering changes in the plant metabolism by affecting plant growth, metabolites biosynthesis, their qualitative and quantitative compositions to a greater extent (Dow et al. 1981). The plants produce various secondary products with an economic value but salinity stress can result in an enhanced production of these metabolites such as isoflavones, isoprenoids, phenols or alkaloids and phytosterols (Khan et al. 2011). Many studies confirmed the negative effect of salinity on secondary plant metabolites. According to Borghesi et al. (2011) results, salinity stress could lead to similar or higher increases in tomato carotenoids and in some cases to a two- to threefold increase in lycopene content as the control. Ayaz et al. (2000) showed that a decrease in germination related to salinity induced disturbance of metabolic pathways leading particularly to an increase in phenolic content. Phenols constitute a part of cellular solutes and provide a reducing environment stress (Sonar et al. 2011). Thus, enhanced synthesis of these secondary metabolites under stressful conditions is believed to protect the cellular structures from oxidative effects (Bourgou et al. 2008). To avoid oxidative

damage resulting from salt stress, higher plants have developed different adaptive mechanisms through the biosynthesis of a cascade of antioxidants. Indeed, polyphenolic compounds participate in the defense against reactive oxygen species (ROS), which are inevitably produced when aerobic or photosynthetic metabolism is impaired by environmental stresses (Ksouri et al. 2007). Such responses depend mainly on the inherent salt tolerance of the plant, the severity of salt stress and the duration of exposure of the plant roots to the salt. Plants employ biochemical and molecular mechanisms to cope with salt stress such as induction of antioxidative enzymes and synthesis of compatible solutes. Plants with salinity tolerance are thought to have mechanisms that allow them to maintain photosynthesis in the presence of high levels of salt (Lee et al. 2004). Contrarily, sensitive responses could result in accelerated senescence and eventually death of the plant (Abogadallah 2010). In this context, detoxification of ROS is considered as an essential component of salt tolerance. It has been proven that, in the hard environmental conditions, the amount of antioxidant and antimicrobial components are increased in the plant tissues (Maisuthisakul et al. 2007). Salt tolerance of most crops varies with the change in stages of life cycle. However, other species revealed that the degree of salt tolerance is maintained at different growth stages (Waheed et al. 2006). The severity of salinity response is also mediated by environmental interactions such as humidity, temperature and radiation (Shannon and Grieve 1999).

Safflower (*Carthamus tinctorius*) is one of the largest and most popular genuses of the Asteraceae family widely used in flavoring and coloring all around the world. Safflower is considered to be a moderately salt-tolerant crop (Dajue and Hans-Henning 1996). The use of saline water on safflower has diverse effects. It has been reported that safflower is more sensitive to salinity at germination than at later growth stages because salinity reduces the rate and percent emergence at levels lower than those affecting plant growth (Francois et al. 1964). Other factors that affect safflower's tolerance to salinity include climate, weather, irrigation, soil conditions and fertility (Kaffka and Kearney 1998). Plant responses to NaCl stress have been studied intensively using anatomical, physiological, molecular and proteomic approaches (Šutković et al. 2011). Whereas, to the best of our knowledge, there is no previous study relative to safflower bioactive compounds production and the capacity of these molecules to scavenge toxic free radicals under salinity stress. Thus, this study reports on one hand the impact of salinity on carotenoid and phenol contents and on the other hand the antioxidant and antimicrobial (antibacterial and antifungal) behaviors in *C. tinctorius* flowers in pot culture and to identify the sensitive growth stage in safflower to salinity stress conditions.

Materials and methods

Chemicals

Solvents (analytical grade quality) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (NaOH), chlorhydric acid (HCl), disodium hydrogen phosphate (Na_2HPO_4), sodium monobasic phosphate ($NaH_2PO_4H_2O$), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), butylated hydroxytoluene (BHT), β-carotene, linoleic acid, ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), iron(II) tetrahydrate, iron(II), iron(III), 1,1-diphenyl-2-picrylhydrazyl (DPPH), polyvinyl polypyrolidone Folin_Ciocalteu reagent, potassium ferricyanide ($K_3Fe(CN)_6$) and aluminum chloride ($AlCl_3$) were purchased from Sigma_Aldrich (Steinheim, Germany). Authentic standards of phenolic compounds were purchased from Sigma and Fluka. Stock solutions of these compounds were prepared in HPLC-grade methanol. These solutions were wrapped in aluminum foil and stored at 4 °C. All other chemicals used were of analytical grade.

Plant material and salt treatment

The present study was carried out in a greenhouse at the Biotechnology Center in Borj-Cedria Technopark, Tunisia. Safflower was propagated by seeds from plants grown in the National Institute of Agricultural Research Tunisia. Seeds of *C. tinctorius* L. were germinated for 7 days in petri dishes moistened with tap water. Seven-day-old seedlings were transferred into four lots in controlled greenhouse conditions at an approximate thermo-period of 30/20 °C (day/night), 60–80 % air humidity, under aeration, and artificial light of 282 Lmol/m^2 s with 16/8 h photoperiod (day/night). Then the growth medium was laced progressively with NaCl during 7 days until reach the four following concentrations: 0, 5, 10, and 15 g/L. These concentrations were kept constant throughout the experiment and added a nutrient solution (Hoagland and Arnon 1950) each week. The salt treatment lasted a month. At the end of this period, salt stress symptoms (leaves chlorosis and necrosis) appeared and flowers were harvested at two flowering stages.

Plant growth and yield components

For each treatment, measurements of plant height, fresh and dry matter weights were evaluated by destructive harvests of six randomly selected plants from the center rows of each pot. Plants were harvested and immediately weighted (fresh matter weight). After that, plants were wrapped in a clean paper bag, labeled and then oven-dried

at 65 °C for 48 h to constant weight and reweighted (dry matter weight). Their dry matter contents were computed using the following equation:

$$DM\ (\%) = DMW/FMW \times 100$$

where DM: dry matter, FMW: fresh matter weight and DMW: dry matter weight. Moreover, for each treatment yield components such as number of flower heads per plant, number of seeds per plant and yield of petals were determined at the end of the experiment.

Determination of total phenolics, flavonoids and proanthocyanidins

Total phenolics

Colorimetric quantification of total phenolics was determined, as described by Dewanto et al. (2002). In brief, 125 μL of suitable diluted sample extract were dissolved in 500 μL of distilled water and 125 μL of Folin-Ciocalteu reagent. The mixture was shaken, before adding 1,250 μL of Na_2CO_3 (7 g/ 100 mL), adjusting with distilled water to a final volume of 3 mL, and mixed thoroughly. After incubation for 90 min at 23 °C in darkness, the absorbance versus a prepared blank was read at 760 nm. Total phenolic content of flower extract was expressed as milligram gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50–400 mg/mL ($R^2 = 0.99$). All samples were performed in triplicate.

Total flavonoids

Total flavonoid content was measured according to Dewanto et al. (2002) An aliquot of diluted sample was added to 75 μL of $NaNO_2$ solution (5 %), and mixed for 6 min, before adding 0.15 mL of $AlCl_3$ (10 %). After 5 min, 0.5 mL of 1 M NaOH solution was added. The final volume was adjusted to 2.5 mL, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm. Total flavonoid content of flower extracts were expressed as milligram catechin equivalents (CE) per gram of dry weight, respectively (mg GAE/g DW) through the calibration curve ranging from 50 to 500 mg/mL ($R^2 = 0.85$).

Total proanthocyanidins

In the presence of concentrated HCl, condensed tannins were transformed by the reaction with vanillin to anthocyanidols (Sun et al. 1998). 50 μL of the extract appropriately diluted was mixed with 3 mL of vanillin (4 %) and 1.5 mL of 12 N HCl. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents of flowers were expressed as milligram catechin equivalents (CE) per

gram of dry weight through the calibration curve with catechin. The calibration curve range was 50–600 mg/mL ($R^2 = 0.85$).

Carotenoid content

The extraction of carotenoids, using a modified procedure from Riso and Porrini (1997), was performed by incubating 500 mg of the flowers together with 10 mL methanol for 1 h in a shaking incubator at 120 rpm. Afterwards, the mixture was homogenized for 30 s in an ice bath and the probe washed with methanol. The mixture was centrifuged at 3,000 rpm for 5 min. The methanol layer was transferred into a 50-mL volumetric flask and the extraction repeated four times with 10 mL of Tetra Hydro Furan (THF), followed by vortexing and centrifugation. The THF layers were combined with the methanol layer and the volume was brought up to 50 mL. One millilitre of the extract was taken, dried under nitrogen and re-suspended in 1 mL of ethanol. Triplet samples of each vegetable were extracted. The carotenoid contents were calculated using the following formula (Kirk and Allen 1965)

$$\text{Carotenoid contents (mg/ 100 g)}$$
$$= A\ 480 \times \text{volume of extract} \times 10 \times 100/2,500$$
$$\times \text{weight of plant material.}$$

Analysis of individual phenolic compounds by analytical RP-HPLC-MS

The HPLC-DAD-MS analyses were performed using an Agilent 1100 Series LC/MSD system with a diode array detector (DAD) coupled to a mass spectrometer (MS) (quadrupole analyser) equipped with an electrospray ionization interface (ESI, Agilent). The separation was carried out on a 250 × 4.6 mm, 4 μm Hypersil ODS C18 reversed phase column. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2 % sulfuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 15 % A/85 % B 0–12 min, 40 % A/60 % B 12–14 min, 60 % A/40 % B 14–18 min, 80 % A/20 % B 18–20 min, 90 % A/10 % B 20–24 min, 100 % A 24–28 min. The injection volume was 20 μL, and peaks were monitored at 280 nm. Mass spectrometry parameters were as follows: capillary voltage 400 V, fragmentor 160 V, drying gas temperature 350 °C, gas flow (N2) 10 L/ min, nebulizer pressure 50 psig. The instrument was operated in positive ion mode scanning from m/z 100 to 800 at a scan rate of 1.43 s/cycle. Peaks were identified by congruent retention times compared with those of authentic standards. Phenolic compound contents were expressed in microgram per gram of dry plant material weight (Salem et al. 2011).

Antioxidant activity

DPPH assay

The electron donation ability of flower extracts was measured by bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al. (1988). Half-a-milliliter of 0.2 mM DPPH methanolic solution was added to flower extracts of *C. tinctorius* (2 mL, 10–1,000 µg/mL). After an incubation period of 30 min at room temperature, the absorbance was read against a blank at 517 nm. The inhibition percentage of free radical DPPH (IP%) was calculated as follows:

$$IP\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. Extract concentration providing 50 % of inhibition (IC_{50}) was calculated from the regression equation prepared from the concentration of the extracts and the inhibition percentage. BHT was used as a positive control ($R^2 = 0.93$).

Reducing power

The method of Oyaizu (1986) was used to assess the reducing power of *C. tinctorius* flower extracts. These extracts (1 mL) were mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide ($K_3Fe (CN)_6$) and incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10 % trichloroacetic acid was added to the mixture that was centrifuged at 650*g* for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % iron (III) chloride solution. The intensity of the blue-green color was measured at 700 nm. The extract concentration at which the absorbance was 0.5 for the reducing power (EC_{50}) was obtained from the linear regression equation prepared from the concentrations of the extracts and the absorbance values. High absorbance indicates high reducing power. Ascorbic acid was used as a positive control ($R^2 = 0.97$).

Metal-chelating power

According to Zhao et al. (2006), 0.1 mL of flower extracts was added to 0.05 mL of 2 mM $FeCl_2$. The reaction was initiated by the addition of 0.1 mL of 5 mM ferrozine and 2.75 mL of distilled water. The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was then measured at 562 nm. The scavenging activity was calculated as follows:

$$IP\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. IC_{50} was calculated from the plot of inhibition percentage against extract concentration. EDTA was used as a positive control ($R^2 = 0.88$).

β-Carotene–linoleic acid assay

Antioxidant capacity of *C. tinctorius* extracts was evaluated by β-carotene–linoleic acid assay. The β-carotene bleaching method is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al. 2004). First, β-carotene (0.2 mg) was dissolved in 1.0 mL of chloroform. Afterward, 200 µL of linoleic acid and 0.2 mL of Tween 80 were added and the mixture was left at room temperature for 15 min. After evaporation of chloroform, 50 mL of oxygenated distilled water was added and the mixture was shaken to form an emulsion (β-carotene–linoleic acid emulsion). Aliquots of 3.0 mL of this emulsion were transferred into test tubes containing 0.2 mL of different extract concentrations. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance (A_0) was measured at 470 nm using a spectrophotometer. A second absorbance (A_1) was measured after 120 min. A_{blank}, without β-carotene, was prepared for background subtraction. Lipid peroxidation (LPO) inhibition was calculated using the following equation (Soares et al. 2009):

$$LPO \text{ inhibition } (\%) = A_1/A_0 \times 100.$$

The extract concentration providing 50 % of antioxidant activity (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHT was used as standard ($R^2 = 0.93$).

Screening of antibacterial and antifungal activities

Antibacterial activity was analyzed by the disc diffusion method (Rios and Recio 2005) against seven human pathogenic bacteria including gram-positive *Staphylococcus metecilin resistante*, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14759, *Salmonella sp* DMB 560, *Listeria monocytogenes* ATCC 19195 and gram-negative bacteria; *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. All bacteria were grown on Mueller–Hinton plate at 30 °C for 18–24 h previous inoculation onto the nutrient agar. A loop of bacteria from the agar slant stock was cultivated in nutrient broth overnight and spread with a sterile cotton swap onto

Petri dishes containing 10 mL of API suspension medium and adjusted to the 0.5 McFarland turbidity standards with a Densimat (BioMérieux). Sterile filter paper discs (6 mm in diameter) impregnated with 20 μL of plant extract (10 mg/mL) was placed on the cultured plates. After 1–2 h at 4 °C, the treated Petri dishes were incubated at 25 or 37 °C for 18–24 h. The solvents: acetone/water, 2:8 and pure methanol without extracts served as negative controls and Gentamycin was used as the positive one. The antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zone around the discs. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded. The same agar-disc diffusion method was used for screening the antifungal activity of *C. tinctorius* flower extracts. Five yeast strains (*Candida albicans*, *Aspergillus niger*, and *Sclerotonia sclerotoria*) were first grown on Sabouraud chloramphenicol agar plate at 30 °C for 18–24 h. Several colonies of similar morphology of the clinical yeast were transferred into Api suspension medium and adjusted to 2 McFarland turbidity standard with a Densimat (Bio-Merieux). The inocula of the respective yeast was streaked onto Sabouraud chloramphenicol agar plates at 30 °C using a sterile swab and then dried. A sterilized 6-mm paper disc was loaded with 20 μL (10 mg/mL) of flower extracts. The treated Petri dishes were placed at 4 °C for 1–2 h and then incubated at 37 °C for 18–24 h. The inhibition of fungal growth was also evaluated by measuring the diameter of the transparent inhibition zone around each disc. The average of three measurements was taken. The susceptibility of the standard was determined using a disc paper containing 300 μg of nystatine.

Statistical analysis

All analyses were performed in triplicate, and the results are expressed as mean values (standard deviations (SD)). The data were subjected to statistical analysis using statistical program package STATISTICA (Statsoft 1998). The one-way analysis of variance (ANOVA) followed by the Duncan multiple range test was employed and the differences between individual means and each solvent used were deemed to be significant at $P < 0.05$.

Results and discussion

Plant growth

Growth analysis is a fundamental characteristic to study plant response to an environmental stress. Growth parameters were measured at the end of salt treatment. As shown in Table 1, significant decreases ($P < 0.05$) in plant growth

were observed following the application of increasing NaCl levels, independently of safflower variety. The application of 5 g/L of NaCl caused a light drop (1.6 and 1 %, respectively, in 104 and Jawhara) of the plant height, while concentrations of 10 g/L and 15 g/L of NaCl reduced plant height by about 16.3 and 27.5 % (104) and 17.4 and 29.3 % (Jawhara), respectively, by referring to control plants. In accordance with our findings, Menadi (1997) also found a significant decrease in safflower plant height by about 65.6 % at 9 g/L of NaCl in respect to the control. Similarly, same findings were observed in sunflower at 12 g/L of NaCl and in Colza (Najine 1996). There are no significant ($P < 0.05$) differences on dry matter between control and treated plants but significant ($P < 0.05$) decreases by 67.9 and 75.7 % (104) and 41.2 and 59.86 % (Jawhara) were observed following the application of 10 and 15 g/L of NaCl, respectively, in comparison with the control. Conversely, with increasing of NaCl levels, the flower head number by plant was significantly reduced (Table 1). Under moderate salinity stress (10 g/L of NaCl), this reduction is more pronounced with 104 (55.6 %) in comparison with Jawhara (45.5 %) with respect to the control. Our findings were in agreement with those reported on American safflower which is considered to be a moderately salt-tolerant crop (Francois et al. 1964). While generally regarded as tolerant, safflower yields also have been reduced. We noted a substantial decline in seeds number in the two varieties and 104 seems to be less affected by salinity level as compared to Jawhara variety. The medium number of seeds is the same for the two varieties (47). The application of 10 and 15 g/L of NaCl reduced significantly seeds number by 28.7 and 48.1 % for 104 and by 29.0 and 60.8 % for Jawhara. In addition, petals yield decreased with increasing of NaCl levels. The reduction was in average of 14.0 % for the two varieties.

Effect of salinity on non-enzymatic antioxidants composition

Polyphenols

Differences in polyphenol, flavonoid and proanthocyanidin contents in *C. tinctorius* flowers at the two studied development stages (full flowering and post flowering) have been observed under salt stress (Fig. 1). In the control, the phenolic content of *C. tinctorius* flower was measured as 34.0 and 34.8 mg gallic acid equivalent per gram of extract in 104 and Jawhara varieties, respectively at full flowering stage (F_1). Concerning post flowering stage (F_2), the phenolic content was measured as 38.1 and 39.4 mg gallic acid equivalent per gram of extract with 104 and Jawhara varieties, respectively. Significant induction of polyphenol accumulation was observed with increase of NaCl

Table 1 Salt effect on *C. tinctorius* plant growth

NaCl (g/L)	Height (cm)		Dry matter weight (g)		Fresh matter weight (g)		Dry matter (%)	
	104	Jaw	104	Jaw	104	Jaw	104	Jaw
0	130.3 ± 0.1[a]	131.1 ± 0.4[a]	313.0 ± 1.6[a]	220.7 ± 0.0[a]	1,350.2 ± 1.3[a]	1,432.7 ± 1.9[a]	23.2 ± 0.0[a]	15.4 ± 0.1[a]
5	128.2 ± 0.0[b]	129.9 ± 0.1[b]	304.1 ± 1.8[b]	217.2 ± 1.1[b]	1,345.8 ± 1.9[b]	1,430.9 ± 1.5[a]	22.6 ± 0.7[a]	15.2 ± 0.1[a]
10	109.1 ± 0.0[c]	108.3 ± 0.1[c]	100.7 ± 1.7[c]	129.7 ± 1.0[c]	875.2 ± 0.1[c]	916.1 ± 1.1[b]	11.5 ± 0.0[b]	14.2 ± 0.1[b]
15	94.4 ± 1.2[d]	92.8 ± 0.0[d]	76.2 ± 1.1[d]	88.6 ± 1.7[d]	767.0 ± 0.1[d]	823.1 ± 1.2[c]	9.9 ± 0.6[c]	12.2 ± 0.2[c]

NaCl (g/L)	Flower head number/plant		Seeds number/flower head		Petals yield (g)	
	104	Jaw	104	Jaw	104	Jaw
0	27.0 ± 0.3[a]	33.0 ± 0.3[a]	48.0 ± 0.2[a]	46.0 ± 1.2[a]	0.8 ± 0.0[a]	0.7 ± 0.0[a]
5	16.0 ± 0.4[b]	24.0 ± 0.4[b]	40.0 ± 0.0[b]	38.0 ± 1.1[b]	0.8 ± 0.0[a]	0.6 ± 0.1[b]
10	12.0 ± 0.7[c]	18.0 ± 1.4[c]	34.0 ± 0.1[c]	32.0 ± 0.1[c]	0.7 ± 0.1[b]	0.6 ± 0.0[c]
15	5.0 ± 0.1[d]	8.0 ± 0.0[d]	25.0 ± 0.0[d]	18.0 ± 0.0[d]	0.6 ± 0.1[c]	0.6 ± 0.1[d]

Values with different superscripts (a–d) are significantly different at $P < 0.05$ (means of three replicates ±SD)

104, Jaw safflower varieties (104 and Jawhara)

concentration. A significant increase of polyphenol accumulation occurred of about 15.0 and 21.5 % (F_1) and about 16.6 and 26.4 % (F_2) at 10 g/L of NaCl, respectively in 104 and Jawhara varieties with respect to the control. Similar results showed an enhancement in total phenolic contents at moderate saline levels in red peppers (*Capsicum annuum*) (Navarro et al. 2006). Some investigators reported the increase in the levels of polyphenol contents under the influence of increasing levels of salinity as a response to the oxidative stress generated by the ROS formation in the hostile environments (salinity, luminosity, water deficit, etc.) (Ksouri et al. 2008). A similar trend was also found in flavonoid concentrations that increased by 34.1 and 34.8 % (F_1) and by 32.7 and 32.9 % (F_2) in 104 and Jawhara varieties, respectively. Concerning proanthocyanidin contents, we noticed an increase of 15.0 and 18.4 % (F_1) and 13.9 and 18.0 % (F_2) with 104 and Jawhara varieties respectively. Flavonoid accumulation is frequently induced by abiotic stress. These compounds promote roles in plant protection against damaging effects (Sonar et al. 2011). This may be due to enzymatic activity inductions occurring under salinity condition and resulting in synthesis of different flavonoid compounds (Haghighi et al. 2012). On the other hand, polyphenol contents showed a decrease at 15 g/L of NaCl estimated by 2.4 folds (F_1) and 1.3 folds (F_2) with the two varieties as compared to the control. Likewise, the flavonoid and proanthocyanidin contents also decreased at severe salinity treatment (Fig. 1). This decrease was of 2.2 (F_1) and 2.0 folds (F_2) for flavonoids and of 1.3 (F_1) and 1.5 folds (F_2) for proanthocyanidins in the two varieties as compared to control plants. Results showed that salinity effect was more pronounced in Jawhara than 104 at the two development

stages (Fig. 1). In fact, the degree to which growth is influenced by salinity differs greatly with species and to a lesser extent with varieties within a species (Shannon and Grieve 1999).

This trend is detected in others members of Asteraceae family such as *Cynara cardunculus* (Falleh et al. 2008) and *Cynara scolymus* (Rezazadeh et al. 2012) grown under osmotic stress. Changes in the contents of phenolics have been associated with the modification of Phenylalanine ammonia-lyase (PAL) activity, which catalyzes the transformation of L-phenyalanine into trans-cinnamic acid. In fact, its activity increases in response to constraint and is considered by most authors to be one of the main lines of cell acclimation against stress in plants (Leyva et al. 1995). In fact, under high levels of salinity, photosynthesis and absorption of phosphor and potassium which are the main substances for secondary metabolites production decreased (Rezazadeh et al. 2012). This may explain the decrease of phenolic content in our results. Phenolic compounds accumulation in plants can be influenced by stress, but this is critically dependent on plants sensitivity (Kim et al. 2007). Based on these results, it seems that moderate salinity stress can induce shikimate pathway in tolerant cultivar. In this context, plants subjected to oxidative damage inherent to salt stress display a potent defense system through increasing of phenolic accumulation (Navarro et al. 2006). Safflower is considered as a moderately salt-tolerant crop (Dajue and Hans-Henning 1996). The response to salinity varies with the sensitivity of crops at different growth stages (Ben Abdallah et al. 2013). Indeed, the varieties (104 and Jawhara) which have shown tolerance at moderate salinity stress, exhibited greater reduction in their phenolic contents at post flowering stage
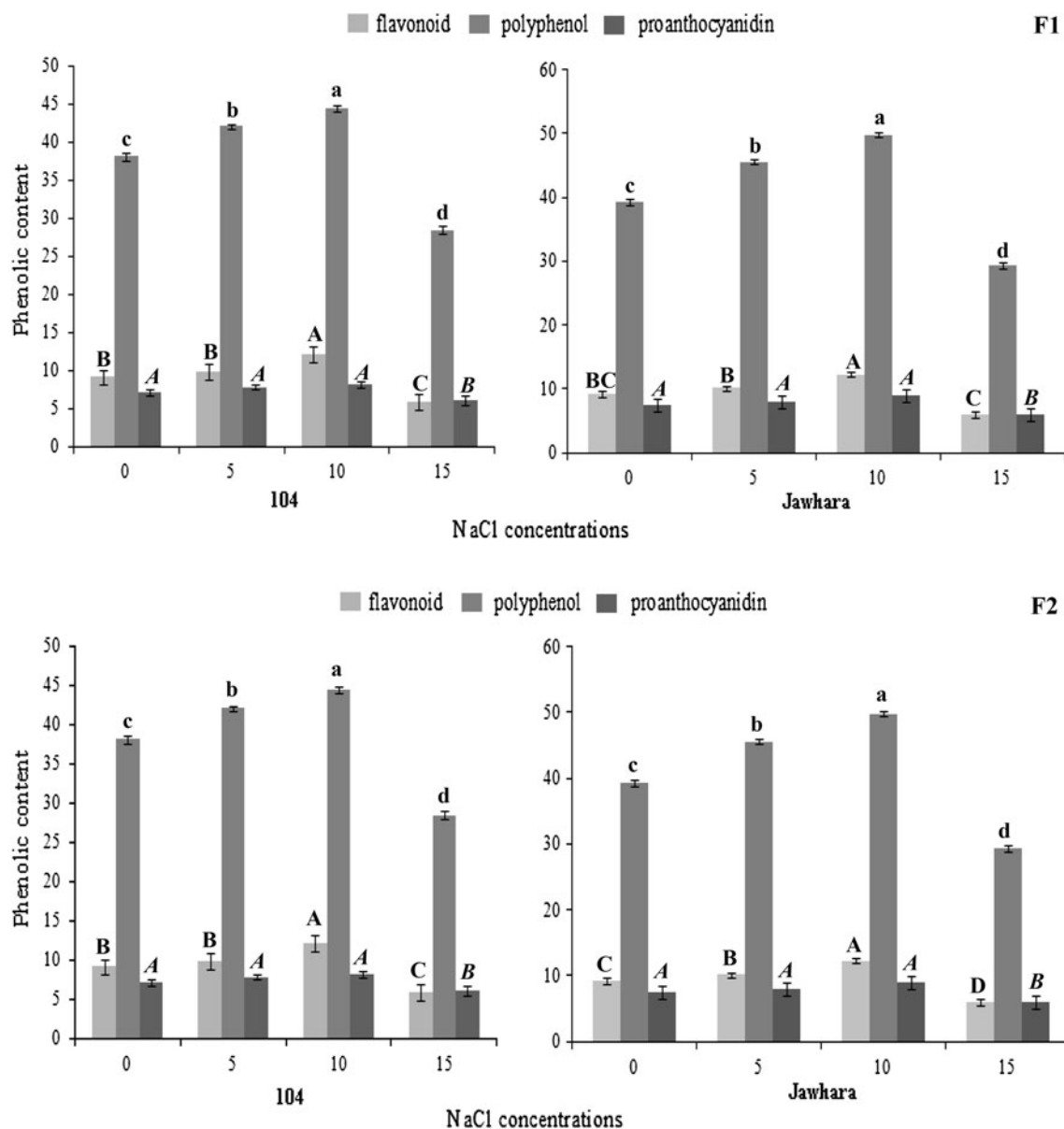
Fig. 1 Total polyphenol, flavonoid, proanthocyanidin contents of *Carthamus tinctorius* flowers at full flowering (F_1) and post flowering (F_2) of 104 and Jawhara varieties influenced by different NaCl levels (0, 5, 10 and 15 g/L). Total phenolic contents were expressed by mg of GAE/g of DW. Total flavonoid and proanthocyanidin contents were expressed by mg of CE/g of DW. Means followed by the same small letter (a–d) did not share significant differences between proanthocyanidin ($P < 0.05$). Means followed by the same capital letter (A–D) did not share significant differences between flavonoid ($P < 0.05$). Means followed by the same capital italic letter (A–B) did not share significant differences between polyphenol ($P < 0.05$)

(Fig. 1). Results show that salt tolerance of safflower varies with stage change where it is more pronounced at post flowering one. In fact, with augmenting of salinity level, the rate of increase in phenol content was more pronounced at post flowering stage than full flowering one especially for proanthocyanidin contents with lesser rate of decrease (Fig. 1). Different physiological, biochemical factors at different stages may be involved.

Phenolic compounds

For a clear understanding of phenolic compounds responses under salt stress, it is necessary to follow changes of these individual compounds in *C. tinctorius* flowers in relation to flower development. Results of Table 2 showed a similar variation in phenolic compounds accumulation for the two varieties as polyphenol and flavonoid contents.

Table 2 Contents of individual phenolic compounds (mg/g DW) of *C. tinctorius* flowers under salt stress

Phenolic compounds	Varieties	NaCl (g/L)							
		0		5		10		15	
		F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Gallic acid	Jawhara	11.1 ± 0.1[d]	12.1 ± 0.2[D]	15.0 ± 0.0[c]	19.0 ± 1.5[C]	22.5 ± 0.7[a]	28.5 ± 1.8[A]	18.2 ± 0.2[b]	23.8 ± 0.0[B]
	104	10.1 ± 0.0[d]	11.4 ± 0.7[D]	13.8 ± 0.3[c]	15.2 ± 0.1[C]	21.9 ± 0.2[a]	25.2 ± 1.2[A]	16.3 ± 0.1[b]	18.0 ± 0.1[B]
Chlorogenic acid	Jawhara	37.3 ± 0.0[c]	42.2 ± 0.3[C]	45.1 ± 1.2[b]	46.6 ± 0.1[B]	74.0 ± 2.1[a]	78.9 ± 1.9[A]	30.1 ± 1.2[d]	32.1 ± 1.3[D]
	104	37.0 ± 1.3[c]	40.4 ± 0.1[C]	40.1 ± 1.7[b]	46.6 ± 1.7[B]	71.1 ± 1.8[a]	74.4 ± 0.0[A]	28.2 ± 0.0[d]	31.1 ± 0.9[D]
Vanillic acid	Jawhara	3.5 ± 0.1[c]	4.1 ± 0.4[C]	4.0 ± 0.1[b]	5.3 ± 0.2[B]	8.0 ± 0.0[a]	9.1 ± 0.3[A]	2.1 ± 0.1[d]	3.2 ± 0.8[D]
	104	3.0 ± 0.1[c]	4.0 ± 0.1[C]	3.9 ± 1.9[b]	4.6 ± 0.6[B]	7.7 ± 0.2[a]	8.4 ± 0.1[A]	2.6 ± 0.1[d]	4.7 ± 0.1[B]
Epicatechin	Jawhara	2.0 ± 1.5[b]	3.0 ± 0.5[C]	2.0 ± 0.3[b]	2.2 ± 0.3[D]	5.6 ± 0.0[a]	6.1 ± 0.1[A]	1.1 ± 0.1[c]	3.7 ± 0.2[B]
	104	1.6 ± 0.0[c]	2.0 ± 0.1[D]	2.2 ± 0.3[b]	3.5 ± 0.1[B]	6.0 ± 0.8[a]	8.0 ± 0.8[A]	1.5 ± 0.3[d]	2.9 ± 0.1[C]
Resorcinol	Jawhara	4.6 ± 1.1[c]	5.8 ± 0.1[C]	7.0 ± 1.8[b]	8.9 ± 0.9[B]	9.0 ± 0.0[a]	10.1 ± 0.0[A]	2.6 ± 0.1[d]	4.6 ± 0.4[D]
	104	4.2 ± 0.1[c]	4.9 ± 0.0[D]	7.0 ± 0.1[b]	7.2 ± 0.5[B]	9.2 ± 0.7[a]	10.0 ± 0.2[A]	2.4 ± 0.2[d]	5.6 ± 0.0[C]
Rutine trihydrate	Jawhara	0.1 ± 0.0[c]	nd	nd	nd	3.1 ± 0.3[a]	4.9 ± 0.0[A]	1.9 ± 0.4[b]	2.3 ± 0.5[B]
	104	nd	nd	nd	nd	3.9 ± 0.1[a]	4.1 ± 0.1[A]	1.0 ± 0.1[b]	1.5 ± 0.1[B]
Quercetin-3-galactoside	Jawhara	14.9 ± 0.2[c]	15.2 ± 0.1[D]	18.7 ± 0.7[b]	19.0 ± 0.1[B]	30.1 ± 1.9[a]	31.2 ± 0.5[A]	13.1 ± 0.7[d]	16.0 ± 0.1[C]
	104	14.9 ± 0.3[c]	16.0 ± 0.0[C]	18.8 ± 0.1[b]	19.1 ± 0.0[B]	27.9 ± 0.0[a]	29.3 ± 0.1[A]	13.0 ± 0.7[d]	13.9 ± 0.0[D]
Quercetin-3-rhamnoside	Jawhara	5.9 ± 0.6[c]	6.1 ± 0.1[C]	6.1 ± 0.0[b]	9.0 ± 0.2[B]	11.2 ± 0.1[a]	11.0 ± 0.3[A]	4.1 ± 0.1[d]	6.0 ± 0.2[D]
	104	5.8 ± 0.8[b]	6.0 ± 0.1[C]	6.0 ± 0.1[b]	7.0 ± 0.2[B]	10.8 ± 0.3[a]	11.9 ± 0.6[A]	3.5 ± 0.6[c]	4.8 ± 0.2[D]
Luteolin	Jawhara	8.1 ± 0.5[c]	9.0 ± 0.4[C]	10.4 ± 0.1[b]	17.7 ± 1.1[B]	20.6 ± 0.3[a]	22.8 ± 0.3[A]	7.2 ± 0.2[d]	6.5 ± 0.4[D]
	104	7.7 ± 0.0[c]	8.0 ± 0.2[C]	10.4 ± 1.0[b]	11.2 ± 0.1[B]	19.3 ± 0.2[a]	20.9 ± 1.0[A]	6.5 ± 0.0[d]	7.7 ± 0.1[D]
Quercetin dihydrate	Jawhara	4.0 ± 0.0[c]	4.5 ± 1.9[C]	5.5 ± 0.1[b]	5.9 ± 0.7[B]	8.1 ± 0.1[a]	9.0 ± 0.1[A]	2.2 ± 0.9[d]	3.3 ± 0.0[D]
	104	4.0 ± 0.0[b]	4.2 ± 0.8[C]	3.9 ± 0.1[c]	6.0 ± 1.0[B]	8.0 ± 0.2[a]	9.1 ± 0.2[A]	2.1 ± 0.1[d]	3.2 ± 0.1[D]

Each value represents mean ± standard deviation of three replicates. Means followed by the same small letter (a–d) did not share significant differences between phenolic compounds at F_1 ($P < 0.05$). Means followed by the same capital letter (A–D) did not share significant differences between phenolic compounds at F_2 ($P < 0.05$)

F_1 full flowering stage, F_2 post flowering stage, *nd* not detected

As salinity increased from 5 to 10 g/L of NaCL, a significant increase in ten main phenolic compounds was observed. Up to the moderate level of salinity in the two varieties, a reduction was observed in phenolic compounds content. Under 10 g/L of NaCL, gallic and chlorogenic acids were the most abundant phenolic compounds reaching their maximum respectively of 22.5 and 74.0 mg/g DW for Jawhara and 21.9 and 71.1 mg/g DW for 104 at full flowering stage (Table 2). In contrast, at higher salinity level (15 g/L of NaCL), a significant decrease of these two phenolic compounds was observed. Therefore, the high antioxidant capacity would be explained by the high content of gallic (Salem et al. 2011) and chlorogenic acids due to their polyhydroxyl natures and caffeoylquinic acid derivatives (Rezazadeh et al. 2012). The results showed a significant variability in phenolic composition behavior between the two varieties under salt stress. In fact, the increase in phenolic compounds contents was more pronounced in Jawhara than 104 (Table 2). The significant increase of gallic and chlorogenic acids accumulation in response to salinity suggests that these phenolic compounds are perhaps stress-induced in safflower. Up to the moderate level of salinity, a reduction was observed in the content of these phenolics. In fact, under salt stress, plant diverts the synthesis of carbohydrates to produce secondary metabolites. But, due to disturbance of enzymatic activities in high salinity, photosynthesis is declined; and production of phenolics will be decreased. Clear differences in individual phenolic contents were detected between the two maturity stages. The major phenolic compounds (especially chlorogenic and gallic acids) under saline conditions were higher at post flowering stage in the two varieties. In contrast, Ben Abdallah et al. (2013) showed that under saline conditions, young leaves of safflower accumulated more polyphenols more than old ones. Hence, polyphenol distribution within plant may reflect different requirements for counteracting abiotic stresses at different growth stages. It can be concluded that moderate salinity induced the normal saline tolerance pathway via increasing total phenolic compounds contents.
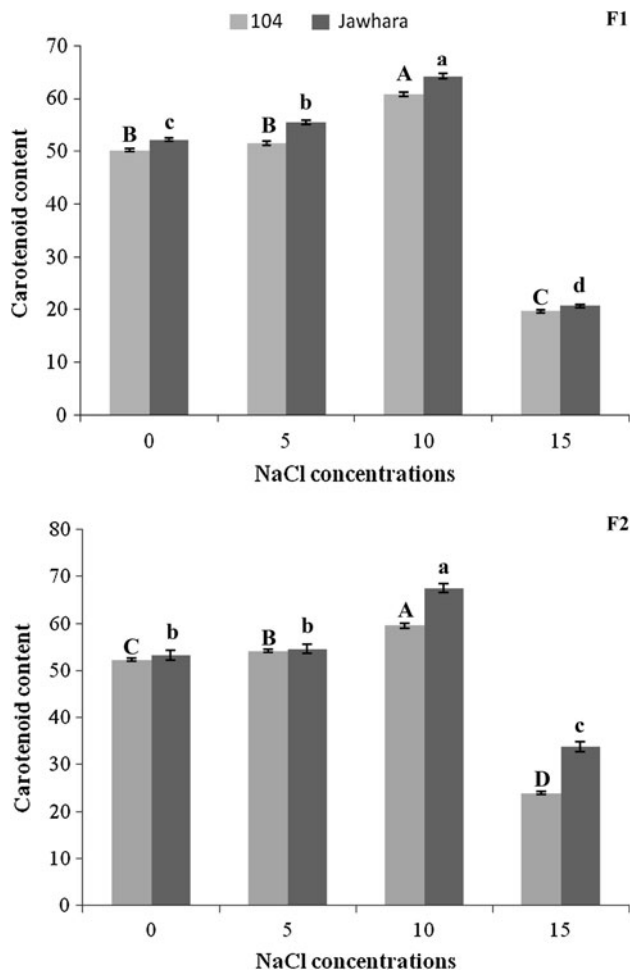
Fig. 2 Effect of salt on carotenoids content of the flowers of two *Carthamus tinctorius* varieties at two flowering stages. Values are mean of three experiments ±SD. Each value is expressed as mg/ 100 g of dry tissue. Means followed by the same small letter (*a–d*, in Jawhara variety) and capital letter (*A–D*, in 104 variety) did not share significant differences (*P* < 0.05)

Carotenoids

Carotenoids are also one of the non-enzymatic antioxidants along with vitamin C, vitamin E and lipoic acid and play an important role in the protection of plants against oxidative stress (Mane et al. 2011). In the present study, maximum increase in the carotenoid content was observed as 17.4 % (104) and 18.8 % (Jawhara) (at 10 g/L of NaCL). However, carotenoid content was drastically reduced by 60.8 % (104) and by 61.3 % (Jawhara) (at 15 g/L) over the control at full flowering stage. Our results were in agreement with those reported on *Vetiveria zizanioides* (Mane et al. 2011) and on *Capsicum Annuum* where the use of moderately high-saline water increased leaf carotenoid contents over the control while higher level induced severe reduction (Ziaf et al. 2009). In fact, at lower levels of salt concentration, carotenoids act as accessory pigments but at higher

levels, these molecules are inhibited and unable to prevent chloroplast from photo-oxidative damage (Mane et al. 2011). The effect of salinity stress on carotenoids was more pronounced at post flowering (the rate of decrease was 1.34 lower) than full flowering stage for the two varieties (Fig. 2). Carotenoids could play a role in preventive anti-oxidative defense (Abogadallah 2010) and their contents may be helpful to differentiate salt sensitive and tolerant cultivars (Ziaf et al. 2009).

Effect of salinity on antioxidative properties

The equilibrium between ROS production and their scavenging may be perturbed by salinity which may lead to significant oxidative damage (Gill and Tuteja 2010). In this study, DPPH and ABTS methods were selected to evaluate the antioxidant activities of plant extracts as they are among the most effective methods for evaluating this activity. All extracts of safflower varieties showed higher levels of scavenging activity on DPPH radicals ranging from (IC_{50} = 1.4 µg/mL) to (IC_{50} = 4.3 µg/mL) than BHT (IC_{50} = 16 µg/mL) (Table 3). However, these extracts showed moderate to high levels of scavenging activity on ABTS radical and lower than Trolox. At full flowering stage, radical scavenging activity showed a gradual increase in flowers by 24.3 and 35.2 % (104) and by 24.4 and 35.6 % (Jawhara) for DPPH radicals under 10 and 15 g/L of NaCl, respectively, in comparison with controls. Concerning ABTS radical cation scavenging activity, an increasing trend was reported and the values varied from 32.1 and 33.2 % (104) and 40.1 and 44.6 % (Jawhara) under 10 and 15 g/L of NaCl, respectively. In fact, various abiotic stresses lead to the overproduction of ROS in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA. For this reason, plants have developed active oxygen-scavenging systems. In this case, we can explain the increase of *C. tinctorius* carotenoid content at post flowering stage as a protective strategy since they are potential quenchers of ROS (Ziaf et al. 2009). A gradual increase was also observed in reducing power assay by 1.8 folds (104) and by 2.2 folds (Jawhara) at 15 g/L of NaCl. Concerning chelating ability and β-carotene–linoleate bleaching assays, although safflower extract showed low ability to chelate iron and prevent the bleaching of β-carotene, with IC_{50} increasing by 1.0 folds (104) and by 1.2 folds (Jawhara) and with EC_{50} increasing by 1.17 folds for the two varieties at 15 g/L of NaCl in comparison to the control. Antioxidant enzymes play important roles in adaptation to stress conditions. Hence, different changes in their activity as a response to salinity were reported in tolerant and sensitive cultivars (Meloni et al. 2003). In addition, the capacity of the antioxidant defense system is often

Table 3 Antioxidant properties of *C. tinctorius* flowers extracts at flowering and post flowering stages under salinity stress

NaCl (g/L)	0		5		10		15	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
104								
IC_{50} (µg/mL) on DPPH	4.2 ± 0.2[a]	3.4 ± 0.1[A]	3.3 ± 1.0[b]	3.4 ± 0.1[A]	3.2 ± 0.2[b]	2.4 ± 0.0[B]	2.7 ± 0.3[c]	1.4 ± 0.6[C]
IC_{50} (µg/mL) on ABTS	389 ± 0.82[a]	306 ± 1.0[A]	304 ± 0.0[b]	280 ± 2.0[B]	264 ± 0.1[c]	256 ± 0.1[C]	260 ± 0.7[d]	245 ± 0.7[D]
EC_{50} (mg/mL) on reducing power	4.5 ± 0.3[a]	0.78 ± 0.19[A]	4.0 ± 0.1[b]	0.7 ± 0.3[B]	2.8 ± 0.1[c]	0.6 ± 0.3[C]	2.6 ± 0.0[d]	0.6 ± 0.0[D]
IC_{50} (mg/mL) on chelating power	14.1 ± 0.1[a]	13.02 ± 0.01[A]	14.0 ± 0.1[b]	12.8 ± 0.1[B]	14.0 ± 0.1[b]	11.8 ± 0.1[C]	13.9 ± 0.0[c]	11.6 ± 0.1[D]
EC_{50} (mg/mL) on LPO inhibition	8.0 ± 0.0[a]	6.88 ± 0.41[A]	7.8 ± 0.3[b]	6.8 ± 0.1[A]	6.8 ± 0.0[c]	5.7 ± 0.1[B]	6.5 ± 0.2[d]	5.5 ± 0.2[B]
Jawhara								
IC_{50} (µg/mL) on DPPH	4.2 ± 0.3[b]	3.90 ± 0.5[A]	4.2 ± 1.2[a]	3.6 ± 0.9[B]	3.2 ± 0.4[c]	2.8 ± 0.2[C]	2.7 ± 1.1[d]	2.5 ± 0.6[D]
IC_{50} (µg/mL) on ABTS	556 ± 1.0[a]	422 ± 1.6[A]	445 ± 0.7[b]	420 ± 1.1[A]	333 ± 0.1[c]	312 ± 0.2[B]	308 ± 0.0[d]	277 ± 0.1[C]
EC_{50} (mg/mL) on reducing power	3.2 ± 0.1[a]	2.0 ± 0.1[A]	2.0 ± 0.0[b]	2.0 ± 0.2[A]	1.6 ± 0.1[c]	0.8 ± 0.1[B]	1.5 ± 0.0[d]	0.8 ± 0.2[C]
IC_{50} (mg/mL) on chelating power	13.2 ± 0.0[a]	11.6 ± 0.1[A]	11.9 ± 0.0[b]	11.4 ± 0.0[B]	11.8 ± 0.1[c]	11.0 ± 0.3[C]	11.1 ± 0.2[d]	11.0 ± 0.0[C]
EC_{50} (mg/mL) on LPO inhibition	9.0 ± 0.1[a]	7.2 ± 0.2[A]	9.0 ± 0.0[a]	6.4 ± 0.2[B]	8.5 ± 0.1[b]	6.4 ± 0.0[B]	8.1 ± 1.5[c]	5.8 ± 0.5[C]

Means followed by the same small letter (a–d) did not share significant differences between antioxidant properties at F_1 ($P < 0.05$). Means followed by the same capital letter (A–D) did not share significant differences between antioxidant properties at F_2 ($P < 0.05$)

F_1 full flowering stage, F_2 post flowering stage

increased under stress conditions (Gressel and Galun 1994) which may be associated with salt tolerance (Abogadallah 2010). Many comparative studies using tolerant plants have correlated the salt tolerance to an increase in the activity of antioxidant enzymes such as in *Catharanthus roseus*, *Gossypium hirsutum* (Meloni et al. 2003) and *Capsicum annuum* (Navarro et al. 2006). Concerning post flowering stage, we found a similar trend where the maximum of the activity was shown with the lowest of IC_{50} and EC_{50}. Radical scavenging activity showed an increase of 2.01 folds (DPPH) and 1.06 folds (ABTS), 4.41 folds for reducing power and 1.19 folds for chelating power and LPO inhibition assays at post flowering stage (Table 3). Therefore, salinity-stressed mature flowers suffer less from stress than salinity-stressed young flowers. This suggests that flower development stage might contribute to differential prevention of oxidative damage in plants exposed to salinity (Telesinski et al. 2008). During flowering stages, Jawhara could be considered as the most tolerant variety against salinity stress than 104 one showing higher phenolic, carotenoid concentrations and antioxidant activity with lower photochemical damage after. According to Rajguru et al. (1999), varietal differences in salt-stress resistance are correlated with differences in antioxidant

enzymes activities. In this study, antioxidant activity did not show correlation with phenol contents. In fact, the synergistic interactions between the antioxidants in the mixture of phenolic compounds make the antioxidant activity dependent not only on the concentration but also on the nature and the interactions between the antioxidants (Djeridane et al. 2006). Based on these results, it seems that plants have evolved complex mechanisms for adaptation to osmotic and ionic stresses caused by high salt. Therefore, a concerted effort to understand the effects of salinity on plants and to develop salt-tolerant species is essential to avoid soil salinization problems.

Effect of water stress on antimicrobial activity

The effect of salinity stress on the antimicrobial activity of *C. tinctorius* flower extracts was investigated. The methanolic extracts of this plant using agar-well diffusion method were tested against five gram-positive bacteria (*E. coli*, *S. aureus*, *B. cereus*, *Staphylococcus Meteciline Resistance* and *Listeria monocytogenes*), two gram-negative bacteria (*Salmonella Sp* and *P. aeruginosa*) and three yeast strains (*C. albicans*, *Aspergillus carbonarus* and *S. sclerotoria*) using agar-well diffusion method. As shown

Table 4 Antibacterial and antifungal activities of *C. tinctorius* flower extracts against seven human pathogenic bacteria and fungi strains at two flowering stages

| | NaCl (g/L) | | | | | | | |
| | 0 | | 5 | | 10 | | 15 | |
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Bacterial strains								
104								
Bacillus cereus ATCC 14759	10.0 ± 0.5[a]	5.0 ± 0.2[A]	9.5 ± 0.2[b]	3.0 ± 0.0[B]	na	2.0 ± 0.1[C]	na	na
Staphylococcus metecilin mesistance	9.5 ± 0.2[a]	5.6 ± 0.1[A]	9.1 ± 0.4[a]	4.8 ± 0.1[B]	8.1 ± 0.5[b]	2.9 ± 0.0[C]	6.1 ± 0.2[c]	2.0 ± 0.2[D]
Listeria monocytogenes ATCC 19195	8.5 ± 0.1[a]	6.7 ± 0.1[A]	7.2 ± 0.4[b]	6.7 ± 0.0[A]	6.9 ± 0.3[c]	5.0 ± 0.0[B]	4.7 ± 0.0[d]	3.8 ± 0.0[C]
E.coli ATCC 25218	9.3 ± 0.0[a]	8.9 ± 0.1[A]	8.3 ± 0.2[b]	8.0 ± 0.1[B]	7.6 ± 0.0[c]	6.2 ± 0.0[C]	5.2 ± 0.1[d]	5.7 ± 0.0[D]
Staphylococcus aureus ATCC 25923	9.8 ± 0.0[a]	9.0 ± 0.0[A]	8.5 ± 0.1[b]	7.8 ± 0.1[B]	7.9 ± 0.1[c]	6.7 ± 0.7[C]	7.7 ± 0.2[c]	5.9 ± 0.1[D]
Pseudomonas aerogenosa ATCC 27853	10.9 ± 0.1[a]	9.7 ± 0.2[A]	10.0 ± 0.0[b]	9.0 ± 0.2[B]	9.0 ± 0.1[c]	8.7 ± 0.1[C]	6.2 ± 0.0[d]	6.0 ± 0.1[D]
Jawhara								
Bacillus cereus ATCC 14759	12.0 ± 0.6[a]	8.0 ± 0.0[A]	7.0 ± 0.0[b]	6.0 ± 0.1[B]	4.0 ± 0.6[c]	3.5 ± 0.2[C]	4.0 ± 0.8[c]	na
Staphylococcus metecilin mesistance	8.0 ± 0.1[a]	5.0 ± 0.1[A]	7.1 ± 0.2[b]	5.0 ± 0.3[A]	7.0 ± 0.3[b]	na	5.0 ± 0.1[c]	na
Listeria monocytogenes ATCC 19195	10.0 ± 0.1[a]	7.0 ± 0.1[A]	9.0 ± 0.4[b]	6.5 ± 0.2[B]	6.0 ± 0.2[c]	6.0 ± 0.1[C]	5.0 ± 0.3[d]	na
E.coli ATCC 25218	11.0 ± 0.0[a]	11.0 ± 0.2[A]	11.0 ± 0.4[a]	9.5 ± 0.3[B]	9.0 ± 0.3[b]	7.5 ± 0.1[C]	7.0 ± 0.1[c]	na
Staphylococcus aureus ATCC 25923	9.0 ± 0.0[a]	7.5 ± 0.0[A]	7.0 ± 0.1[b]	6.2 ± 0.0[B]	7.0 ± 0.1[b]	na	6.0 ± 0.2[c]	na
Pseudomonas aerogenosa ATCC 27853	10.0 ± 0.1[a]	10.0 ± 0.2[A]	7.0 ± 0.0[c]	5.9 ± 0.8[B]	8.0 ± 0.0[b]	5.1 ± 0.1[C]	6.0 ± 0.0[d]	na
Yeast strains								
104								
Candida albicans ATCC 14759	4.9 ± 0.1[a]	4.00 ± 0.22[A]	3.8 ± 0.0[b]	3.8 ± 0.9[B]	2.90 ± 0.1[c]	na	2.0 ± 0.1[d]	na
Aspergillus carbonarus	7.0 ± 0.3[a]	6.90 ± 0.11[A]	6.5 ± 0.1[b]	6.0 ± 0.0[B]	6.0 ± 0.2[c]	5.8 ± 0.2[C]	5.0 ± 0.0[d]	na
Sclerotonia sclerotoria	na	na	na	na	na	na	na	na
Jawhara								
Candida albicans ATCC 14759	5.0 ± 0.2[a]	3.0 ± 0.1[A]	5.0 ± 0.0[a]	2.9 ± 0.2[B]	5.0 ± 0.0[a]	2.0 ± 0.2[C]	4.0 ± 0.0[b]	na
Aspergillus carbonarus	6.0 ± 0.2[a]	5.8 ± 0.2[A]	4.2 ± 0.1[b]	5.0 ± 0.0[B]	4.0 ± 0.0[c]	5.0 ± 0.1[C]	4.0 ± 0.0[c]	na
Sclerotonia sclerotoria	3.0 ± 0.1	na	na	na	na	na	na	na

Results are the mean of three replications; 200 μg/disc, tested at 30 μg/disc. Means followed by the same small letter (a–d) did not share significant differences between antimicrobial properties at F_1 ($P < 0.05$). Means followed by the same capital letter (A–D) did not share significant differences between antimicrobial properties at F_2 ($P < 0.05$). The diameter of disc was 6 mm

F_1 full flowering, F_2 post flowering, *na* no antimicrobial activity (–), inhibition zone <1 mm, *weak inhibition zone* inhibition zone 1 mm, *slight antimicrobial activity* inhibition zone 2–3 mm, *moderate antimicrobial activity* inhibition zone 4–5 mm, *high antimicrobial activity* inhibition zone 6–9 mm, *strong antimicrobial activity* inhibition zone >9 mm

in, Table 4 data analysis revealed that methanolic extracts of Jawhara exhibited higher rates of antibacterial activity than 104 against *B. cereus*, *L. monocytogenes* and *E. coli* (inhibition zone diameters varying between 3 and 12 mm). While, no inhibitory effect was observed against *Salmonella Sp* and *S. sclerotoria* both for Jawhara and 104 varieties respectively. As for salinity effect on antimicrobial activity, this stress reduced significantly ($P < 0.05$) the antimicrobial activity of safflower extracts either with Jawhara or 104. As shown in Table 4, we demonstrated that salinity effect was more pronounced at post flowering stage than full flowering one. However, our results were in

disagreement with the study of Maisuthisakul et al. (2007) who showed that in the hard environmental conditions, the amount of antioxidant and antimicrobial components are increased in the plant tissues. Cano et al. (2008) can explain this decrease by the reduction of bioactive substances amount in the flowers which in turn affected the ability of the extracts to inhibit pathogenic activity, thus affecting the efficacy of the plants as medicinal plants. Therefore, antibacterial activity was determined by a mixture of factors including hydrogen bonding parameters, water solubility and molecular size (Griffin et al. 2000). This suggests that safflower is more sensitive to salt at full flowering stage than post flowering stage.

Conclusion

The application of salt concentrations in the medium showed that *C. tinctorius* could adapt to salt stress at the threshold of 10 g/L of NaCl through deploying physiological and chemical mechanisms. The increase of phenolic biosynthesis at concentrations of 10 g/L concomitant to enhancement of antioxidant activity suggested a better protection of *C. tinctorius* against oxidative damage at this salt level. However, successful plant production under salt stress conditions requires an adequate understanding of salinity affect on *C. tinctorius* performance. Safflower tolerance at moderate salt level may constitute an effective way to significantly enhance its quality for both medicinal and nutritional of bioactive molecule source.

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