Kandil AS *et al.* (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 2, Article number 13110
DOI:10.15835/nbha51213110
Research Article

The correlation of *in vitro* antioxidant potentials with the various biochemical responses of salinized basil leaves

Abeer S. KANDIL[1]*, Mahmoud A. MOHAMED[1],
Hossam S. EL-BELTAGI[2]* Hanan S. GABALLA[1]

[1]*Cairo University, Faculty of Agriculture, Biochemistry Department, Gamma St, Giza 12613, Egypt; abeer.sayed@agr.cu.edu.eg (*corresponding author); elcamel.mahmoud@agr.cu.edu.eg; hanansaid2010@yahoo.com*
[2]*King Faisal University, College of Agriculture and Food Sciences, Agricultural Biotechnology Department, Al-Ahsa 31982, Saudi Arabia; helbeltagi@kfu.edu.sa (*corresponding author)*

Abstract

One of the environmental sustainability issues is salinity. Basil seedlings (*Ocimum basilicum* L.) were treated using NaCl solutions of three different concentrations prepared using irrigation (40, 80, and 130 mM), and various biochemical analyses were performed on basil leaves. The number of leaves, leaf area, moisture, weights, and MDA content of basil decreased significantly as salinity levels increased from 40 to 130 mM; however, dry matter increased. As well, the current study investigated a significant increase in osmolytes (including total soluble sugars and proline) and Na^+ contents. The highest activities of CAT and SOD in the leaf tissues of basil were recorded after treatment with 130 mM NaCl, whereas the polyphenol and total flavonoid contents were negatively influenced. On the other hand, the highest ABTS scavenging activity was observed in the 40 mM-treated leaves at a concentration of 1000 µg/mL; however, the DPPH scavenging potential increased significantly in the 80 mM-treated leaves at 3000 µg/mL. Furthermore, the correlation between *in vitro* antioxidant potentials and biochemical responses was described. A strong correlation was identified between the *in vitro* antioxidant capacities of salinized *O. basilicum* leaves and SOD activity, total flavonoids, and the presence of phenolic acids, particularly *p*-hydroxybenzoic and *o*-coumaric acids at various concentrations. As a result, this is the first study to explain how basil may resist salinity by producing specific antioxidant compounds; therefore, our research recommends use of salinity issue to obtain a better plant material for producing dietary supplements or herbal drugs.

Keywords: antioxidant compounds; biochemical responses; correlation; *in vitro* antioxidant potentials; salinized basil leaves

Introduction

Salinity is a main abiotic stress that noticeably disturbs crop production globally. Annually, approximately 1.5 million hectares of cropland are excluded owing to high salinity. In addition, irrigation water containing traces of NaCl increase the salinity levels of nearby arable soil (Tester and Davenport, 2003).

Received: 01 Mar 2023. Received in revised form: 21 Mar 2023. Accepted: 08 May 2023. Published online: 07 Jun 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Even though salinity negatively affects plant growth by directly damaging and inhibiting it and its effect depends on the salinity of the environment and the salinity tolerance of the plant, saline groundwater is still widely used to irrigate plants that are grown in areas with poor water supply (Abbas *et al*., 2021; Osakabe *et al.*, 2014; El-Beltagi *et al*., 2022a).

Salinity generally induces osmotic stress in plants by decreasing water availability to the roots and increasing Na^+ and Cl^- ion accumulation, which consequently alters various physiological processes of the plant, including the transpiration rate, stomatal conductivity, photosynthesis, and relative growth rate (Caliskan *et al*., 2017; Negrao *et al*., 2017). Reactive oxygen species (ROS) is regenerated under salinity conditions; the plant overcomes the deterioration of ROS by increasing antioxidant defense including catalase (CAT) and superoxide dismutase (SOD) (Jabeen and Ahmad, 2013; Sevindik *et al*., 2017). Enhanced salinity tolerance in plants can be attributed, at least partially, to the activity of antioxidant enzymes present in the leaves, such as peroxidase and catalase (CAT), which are considered the primary salinity sensitive enzymes, and proline can induce an increase in these activities (Nawaz and Ashraf, 2010).

Furthermore, malondialdehyde (MDA) levels can be used as a marker of the degree of salinity-induced oxidative stress (Jakovljević *et al*., 2017); it plays a vital role in regulating the properties of membrane lipid compounds in response to the deleterious effects of osmotic stress. Since salinity particularly enhances ROS formation, it consequently affects the proportion of secondary compounds, such as phenolic compounds, essential oils, and photosynthetic pigments (Talebi *et al*., 2018; de Azevedo Neto *et al*., 2019; Scagel *et al*., 2019). Basil (*Ocimum basilicum* L.; Lamiaceae) grows abundantly throughout the Mediterranean, especially in warmer areas. *Ocimum* spp. are known to contain high quantities of bioactive compounds with several medicinal, nutritional, and pharmaceutical applications. For example, *O. basilicum* is one of the chief species from which essential oils are obtained. The essential oils obtained from *O. basilicum* vary in composition, and hence, are used for various applications, such as in condiments, as flavouring agents, and even in folk medicine for treating mental fatigue, dysentery, nausea, rhinitis, and cold (Tarchoune *et al*., 2012; Ramadan *et al*., 2022a).

O. basilicum also contains beneficial bioactive compounds, including phenolic acids (e.g., rosmarinic, caftaric, vanillic, *p*-coumaric, benzoic, hydroxybenzoic, syringic, ferulic, protocatechuic, and chicoric acids, flavonoids (e.g., flavonol-glycosides, rutin, and isoquercetin) (Vlase *et al*., 2014), and terpenoids (e.g., methyl chavicol and linalool) (Alkuwayti *et al*., 2020), which are considered to have the ability to prevent chronic diseases and engender antifungal, antioxidant, anticancer, antimicrobial, and immuno-stimulatory effects (Zabka *et al*., 2014; Rizvi *et al*., 2012). Moreover, the previous studies showed powerful applications involving (anticancer, antibacterial, and biological activities) of phenols and flavonoids which derived of natural sources (Mohammed *et al*., 2018; El-Beltagi *et al*., 2022b). However, little is known regarding the positive correlation of *in vitro* antioxidant potentials with the various biochemical responses of *O. basilicum* under NaCl stress. Hence, the current study designed to evaluate the biochemical responses of *O. basilicum* leaves under NaCl pressure and investigate the possible pathway for tolerance salinity stress.

Materials and Methods

Authentication and experimental design

O. basilicum seeds were authenticated at the Flora and Phyto-Taxonomy Research Department of the Agricultural Research Centre, Dokki-Cairo, Egypt. The study was conducted in 2020 and 2021 in a greenhouse at the Faculty of Agriculture, Cairo University, Egypt. The experiments were performed as described here using a completely randomized design and 30 replicates for each treatment. The soil and water used were first examined according to a method previously reported by Estefan *et al.* (2013). In April, 25-day-old seedlings were transferred into pots (diameter, 20 cm; height, 18 cm) packed with soil (pH = 7.86 and electric

conductivity (EC) = 2.11 ds/m). When the seedlings were 45 days old, three NaCl solutions with different concentrations (40, 80, and 130 mM) were prepared by dissolving NaCl in the irrigation water (pH = 7.54 and EC = 0.28 ds/m) and supplied to the plants. The pots were kept at a greenhouse (27 °C and 17 °C day and night air temperatures, respectively). Overall, eight irrigation treatments were applied, and the plants were harvested 25 days following NaCl treatment. In the current study, the treatments were designated as C (Control), S1 (40 mM), S2 (80 mM), and S3 (130 mM).

Chemicals and reagents

All solvents and kits used were of analytical grade and sourced from Sigma-Aldrich (St. Louis, MO, USA).

Determination of O. basilicum leaf area and weight

O. basilicum leaves were harvested and the leaf area was determined using Image J (National Institute of Health, USA)(Schneider *et al.,* 2012). In addition, the fresh leaves were weighed (FW), after which the leaves were dried at 105 °C for 24 h before measuring their dry weight (DW).

The percentage (%) of moisture and dry matter (DM) contents of the leaves were calculated using the following formulas (Arunachalam and Parimelazhagan 2014; Mehdizadeh *et al.,* 2019):

Moisture (%) = $[(FW - DW)/FW] \times 100$

DM (%) = $(DW/FW) \times 100$

Where DW is the dry weight and FW is the fresh weight.

Determination of proline content

Fresh leaves (0.5 g) were homogenized in 10 mL of aqueous sulfosalicylic acid (3%) and filtered through a Whatman #2 filter paper. Next, 2 mL of the filtrate was added to a mixture of 2 mL of ninhydrin and 2 mL of glacial acetic acid. The mixture was then incubated at 100 °C for 1 h before cooling. Next, 4 mL of toluene was added to the mixture and mixed. The toluene fraction was measured at 520 nm using a spectrophotometer, Jenway, England. The concentration of proline was determined by interpreting a standard curve (R^2= 0.9916) (Bates *et al.,* 1973).

Determination of total soluble sugar content

Fresh leaves weighing 0.2 g were soaked in methanol (80%) with refluxing for 6 h at 80 °C. Then, 2 mL of the solution was added to 0.05 mL phenol (80%), to which 5 mL of H_2SO_4 conc. was added. The tubes were incubated at 30 °C for 20 min. The absorbance of reaction was measured at 490 nm (for hexose) and 480 nm (for pentose and uronic acid) against a blank. The concentrations of the sugars were calculated using the standard curve (R^2= 0.991) (Dubois *et al.,* 1956).

Determination of Na^+ and K^+ ion concentrations

One gram of leaves was added to a mixture of 7 mL of H_2SO_4 and 2 mL of H_2O_2 in a digestion tube placed on a temperature-controlled hotplate. The samples were homogenized by gently swirling the solution, and the resulting homogenate was heated until clear. The solution was then cooled and transferred to a volumetric flask before filtration. The levels of Na^+ and K^+ in the solution were detected by iCE 3300 Atomic Absorption Spectrometer (Thermo Scientific, Germany)(Christian 1970).

Determination of antioxidant enzyme activities and MDA content

Preparation of enzyme extract

We homogenized 500 mg of *O. basilicum* leaf tissue in a mixture comprising 3 mL sodium phosphate buffer (100 mM, pH 7, polyvinylpyrrolidone [1%], and EDTA [1 mM]). The centrifugation of resulting

homogenate was at 7000 rpm for 20 min under cooling. The supernatant was stored till further analyses at ⁻80 °C.

Determination of CAT activity

CAT activity was investigated as described Aebi (1984). CAT activity was expressed in U/ mg protein, where each unit of CAT reacts with H_2O_2 (1 µM, pH 7.0)/min at 25 °C (Aebi 1984).

Determination of SOD activity

The reaction was developed by adding the subsequent reagents: 50 µL enzyme extract, 200 µL of nitroblue tetrazolium (NBT) (1.125 mM), 200 µL of methionine (195 mM) ,100 µL of EDTA (0.3 mM), 200 µL of riboflavin (60µM), and 2.4 mL of sodium phosphate buffer (50 mM, pH 7.8).

The mixture was incubated for 10 min under fluorescent light. The absorbance was read at 560 nm. One unit of SOD was described as the extent of enzyme that inhibits 50% of the NBT reduction under the assessment condition (Giannopolitis and Ries, 1977).

Determination of MDA content

Equal volumes of the extract and a mixture of thiobarbituric acid (TBA) (0.5%) and trichloroacetic acid (TCA) (20%) were incubated at 95 °C for 30 min. The absorbance of reaction was measured at 532 nm (Stewart and Bewley, 1980).

Extraction of O. basilicum leaves

The leaves of *O. basilicum* were left at 37 °C for three days until dry, and then a fine powder of air-dried leaves was soaked in methanol overnight. The extracts were filtered through a Whatman filter paper and evaporated using a rotary dryer at 45 °C.

Estimation of total polyphenol content

Polyphenol content was determined as previously described by Prior *et al*. (2005) with some adjustments. Briefly, 125 µL of the methanolic extract was diluted to 400 µL of distilled water, and then 125 µL of Folin–Ciocalteu reagent (diluted 1:1) was added to solution. The resulting mixture was incubated for 3 min at room temperature; following which 250 µL of Na_2CO_3 (7%) was added. The reaction was stored for 30 min at room temperature in the dark. The absorbance was measured at 760 nm. The gallic acid standard was prepared (10–50 mg/L) (R^2=0.9713). The total polyphenol content was calculated as mg of gallic equivalent [GAE]/g of dry leaves (Prior *et al*., 2005).

Estimation of total flavonoid content

Briefly, 0.5 mL of methanolic was added to 0.5 mL of $AlCl_3$ (2%). An orange-yellowish color was obtained after 1 h and absorbance was measured at 420 nm. The quercetin standard (10–100 mg/L) was prepared (R^2=0.9728). The total flavonoid content was calculated as mg of quercetin equivalent [QE]/g of dry leaves (Ordonez *et al*., 2006).

In vitro antioxidant potentials

DPPH scavenging potential

The radical scavenging potential of the methanolic extract was determined based on 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging test (Sharma and Bhat, 2009). The butylated hydroxyanisole (BHA) was used as a positive control (R^2= 0.9993). Briefly, three concentrations (1000, 2000, and 3000 µg/mL) of the methanolic extract or BHA solution (20 µL) were added to 1 mL of DPPH solution (50 µM). The absorbance of reaction was measured at 515 nm after 20 min. Then, DPPH radical scavenging potential was determined

as follows: (%) DPPH scavenging potential = [(absorbance control – absorbance sample)]/(absorbance control) × 100

ABTS scavenging potential

To produce ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cations (ABTS• +), ABTS solution (7 mM) was reacted with potassium persulfate (2.45 mM). Next, this mixture was left for 12–16 h, and the ascorbic acid was used as a positive control (R^2=0.9381). Then, 10 µL of methanolic extract or ascorbic acid solution (at concentrations of 1000, 2000, and 3000 µg/mL) was added to 1 mL of diluted $ABTS^{\bullet+}$ solution. The absorbance of reaction was read at 734 nm after 7 min (Re *et al*., 1999). The $ABTS^{\bullet+}$ scavenging potential was determined as follows:

(%) ABTS scavenging potential = [(absorbance control – absorbance sample)]/(absorbance control) × 100

HPLC analysis

HPLC separation was executed by Agilent 1260 Infinity Series HPLC system (Agilent, USA). A ternary linear elution gradient was used with the following components in the mobile phase: (A) HPLC-grade water and 0.2% H_3P0_4, (B) methanol, and (C) acetonitrile. A flow rate of elution was conducted at 1 ml/min at 38% relative humidity and 25 °C. Then, 20 µL of methanolic extract was injected into Kinetex®5µm EVO C18 fitted with a 100 × 4.6 mm column (Phenomenex, USA). Detection was conducted at 284 nm using a variable wavelength detector. The sample compounds were identified using external calibration curve of a mixture standard phenolic acids and/or flavonoid compounds.

Statistical analyses

All data are described by mean (n = 3) ± standard error (SE). SE and correlation were calculated using Microsoft Excel. The statistical significance of the differences among the mean values was measured via one-way ANOVA by Web Agri Stat Package 2.0 software. *(https://ccari.icar.gov.in/wasp/index.php*). Different letters designate statistically significant differences between the means at ($p < 0.05$).

Results

Appearance and biochemical analyses of basil leaves under salinity stress

The number, area, and moisture content of the leaves were 41.84, 61.60, and 4.64% lower, respectively, in S3-treated leaves compared with control leaves. However, the increasing levels of salinity also increased the DM of the leaves, with S1-treated leaves showing a greater increase (with 59% increase) compared with control leaves (Table 1).

Table 1. Appearance, weights, and the percentages of moisture and dry matter of basil leaves under NaCl stress

Parameters	Treatments			
	C	S1	S2	S3
No. of leaves / plant	18.33 ±1.85 ^{a}	15.33±0.33 ^{ab}	13.33±0.33 ^{bc}	10.66±0.88 ^{c}
Leaf area (cm^2)	10.34 ± 0.33^{a}	4.83 ±0.41 ^{c}	7.49±0.91 ^{b}	3.97±0.19 ^{c}
Fresh leaves weight (g/plant)	1.553±0.067^{a}	0.537±0.01 ^{c}	0.856±0.02 ^{b}	1.012±0.17 ^{b}
Dry leaves weight (g/plant)	0.138±0.00^{a}	0.066±0.00 ^{c}	0.088±0.00 ^{bc}	0.125±0.02^{ab}
Moisture (%)	92.17±0.23 ^{a}	87.54±0.97 ^{c}	89.46±0.66 ^{b}	87.89±0.32 ^{c}
Dry matter (%)	7.83±0.23 ^{c}	12.45±0.97^{a}	9.83±0.50 ^{bc}	12.10±0.32^{ab}

All values represented as mean ± SE (n=3) and comparison of treatment means with ($p < 0.05$).
The results represented average of two seasons. C (Control), S1 (40 mM), S2 (80 mM), and S3 (130 mM).

Figure 1, A shows a decrease in MDA content in response to treatments with higher NaCl concentrations, with non-significance for S1 and S2 treated basil leaves. Conversely, the proline and total soluble sugars contents increased significantly at three NaCl treated basil leaves, compared to control leaves, with S3-treated leaves increasing by 2 folds in sugar content (Figure 1, B and C).

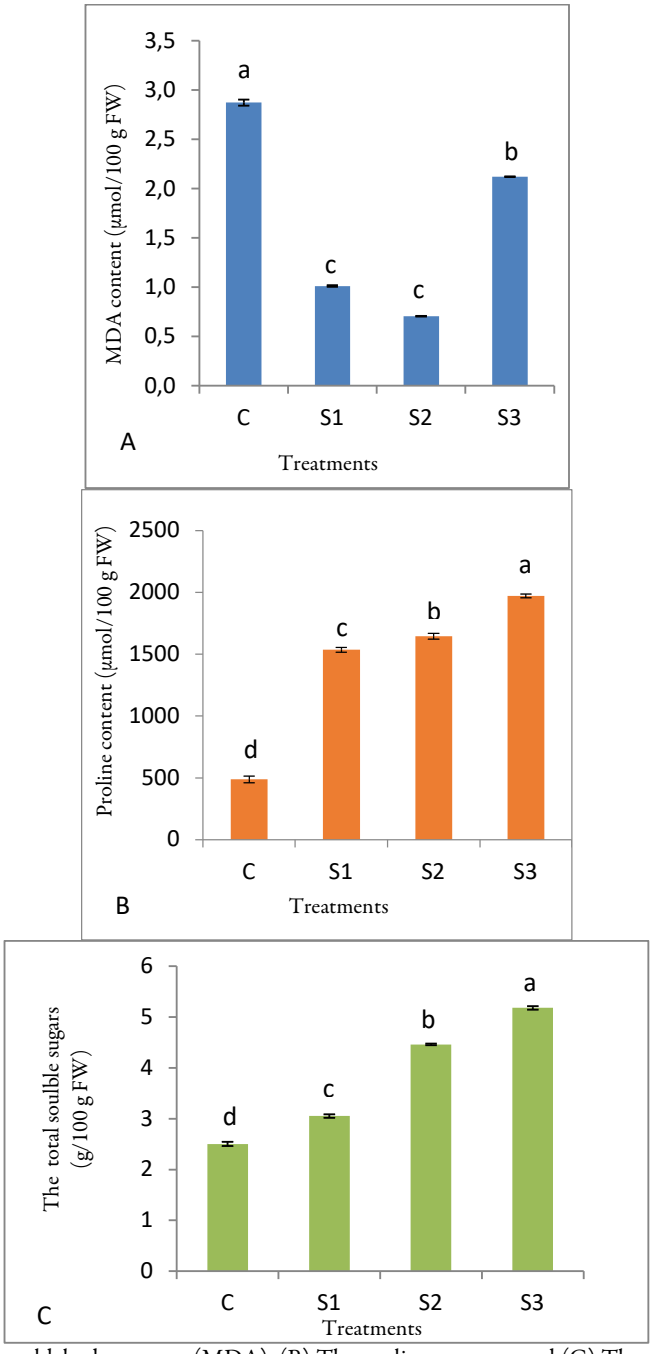


Figure 1. (A) malonaldehyde content (MDA), (B) The proline content, and (C) The total soluble sugars content of basil leaves after NaCl stress

All values represented as mean (n=3) and comparison of treatment means with ($p < 0.05$). The results represented average of two seasons. C (Control), S1 (40 mM), S2 (80 mM), and S3 (130 mM).

The Na^+ and K^+ ion concentrations exhibited significant differences between the NaCl-treated and control *O. basilicum* leaves, with S3-treated leaves increasing by 9.5 folds in Na^+ content, in response to NaCl treatments (Figure 2).

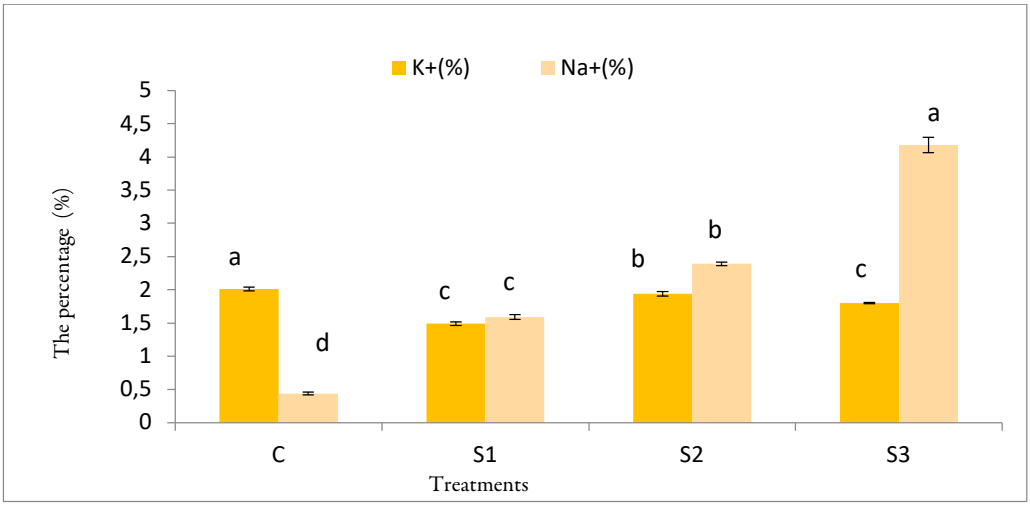


Figure 2. The percentage of potassium and sodium of basil leaves under NaCl stress
All values represented as mean (n=3) and comparison of treatment means with ($p < 0.05$). The results represented average of two seasons. C (Control), S1 (40 mM), S2 (80 mM), and S3 (130 mM).

Effect of salinity stress on antioxidant defense outlines of O. basilicum leaves

Although the highest polyphenol content was observed under moderate NaCl concentrations (S2), S1, S2, and S3-treated leaves showed decreased flavonoid content (by 8.33 and 12.01, and 23.02 %, respectively). However, the polyphenol content remained unaffected at 40 and 80 mM of NaCl concentrations, S3-treated leaves displayed a decrease by 21.95 %. Furthermore, the CAT and SOD activities were displayed an increase by 5.26 and 45%, respectively for S3-treated leaves (Table 2).

Table 2. Antioxidant defense outlines of basil leaves under NaCl stress

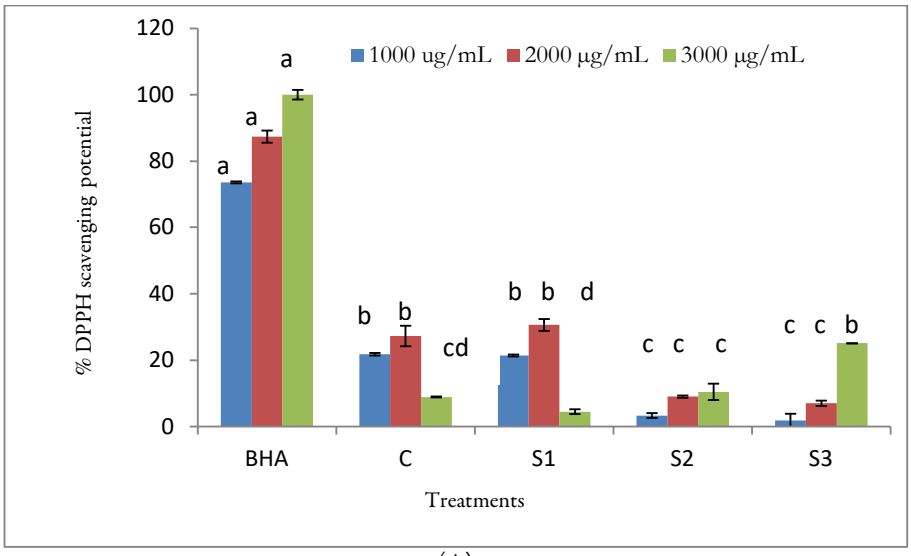
Parameters	Treatments			
	C	S1	S2	S3
CAT (U/mg protein)	89.72±0.27 ^{b}	91.18±0.07 ^{b}	87.48±0.66 ^{c}	94.46±0.10 ^{a}
SOD (U/mg protein)	15.51±0.60 ^{b}	17.66±0.54 ^{b}	20.81±0.57 ^{a}	22.49±0.57 ^{a}
TPC (mg GAE/gDW)	81.71±4.91 ^{a}	86.39± 7.09 ^{a}	94.86±4.04 ^{a}	63.77±2.30 ^{b}
TFC (mg QE/gDW)	30.96±0.44 ^{a}	28.38±0.23 ^{b}	27.24±0.24 ^{b}	23.83±0.48 ^{c}

All values represented as mean ± SE (n=3) and comparison of treatment means ($p < 0.05$).
CAT: Catalase, and SOD: Superoxide dismutase, DW: Dry Weight, GAE: Gallic Acid Equivalent,
QE: Quercetin Equivalent, TPC: Total polyphenol content, TFC: Total flavonoid content.
The results represented average of two seasons. C (Control), S1 (40 mM), S2 (80 mM), and S3 (130 mM

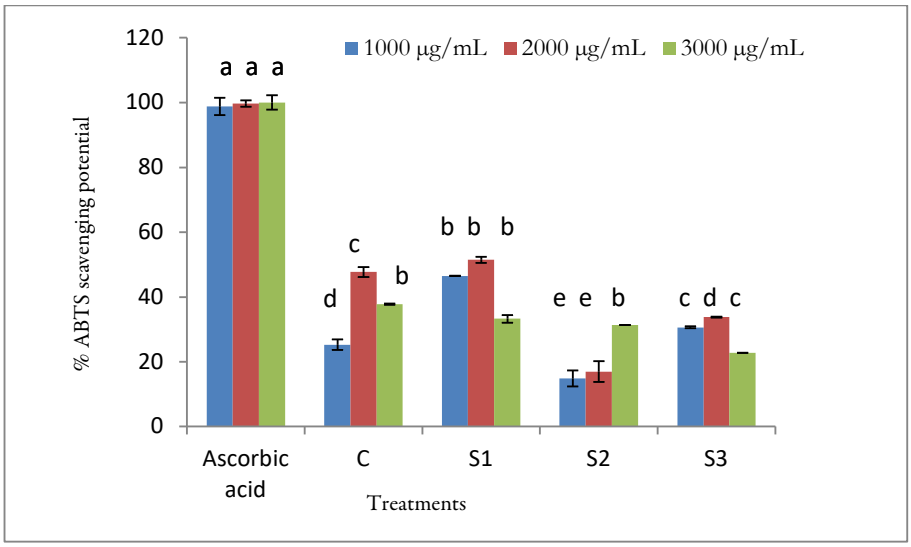
In vitro antioxidant potentials

The antioxidant capacity of *O. basilicum* leaves was measured using DPPH and ABTS assays. A substantial increase in DPPH scavenging activity was observed at concentrations of 1000 and 2000 µg/mL in the control and S1-treated leaves, respectively. When salinity was increased from 80 to 130 mM NaCl, DPPH scavenging potential increased significantly by 17.62% in S2-treated leaves at 3000 µg/mL, while S3 treated leaves investigated 1.8 folds of scavenging at the same concentration (Figure 3, A). However, the highest ABTS scavenging activity was observed in the S1- treated leaves, showing 84.23 and 7.77 % at 1000 and 2000 µg/mL, respectively, while S3-treated leaves increased by 21.31 % at 1000 µg/mL (Figure 3, B). The IC_{50} of control and

NaCl- treated basil leaves for two antioxidant assays were compared and S1-treated leaves was displayed the lowest value in ABTS assay (1037.63 µg/mL).



(A)



(B)

Figure 3. (A) % DPPH scavenging potential, (B) % ABTS scavenging potential of basil leaves under NaCl stress. BHA: (Butylated hydroxyanisole) and ascorbic acid as a positive control, DPPH: 2,2-diphenyl-1-picrylhydrazyl and ABTS: 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
All values represented as mean ± SE (n=3) and comparison of treatment means with ($p < 0.05$). The results represented average of two seasons. C (Control), S1 (40 mM), S2 (80 mM), and S3 (130 mM).

Effect of salinity stress on phenolic acids and flavonoids abundance

The results of our HPLC analysis of the *O. basilicum* leaf methanolic extracts are shown in Table 3. Several phenolic acids and flavonoids were identified, including *p*-hydroxybenzoic acid, quercetin, benzoic acid, ferulic acid, *o*-coumaric acid, kaempferol, and syringic acid. Interestingly, myricetin was a major compound present in both control and treated *O. basilicum* leaf extracts, showing an increase in abundance by 70.20% in

S2-treated leaves. Although, *o*- coumaric acid was displayed a decrease with 90.84% in S3-treated basil leaves, rutin and resveratrol were observed to be highly abundant. Moreover, *p*-hydroxyenzoic acid content declined with increasing salinity. Concurrently, S1 was associated with decreases in the contents of benzoic acid and quercetin by 96.27 and 87.12%, respectively. Conversely, ferulic acid content increased by 178.50, 65.74, and 39.08% in S1, S2, and S3-treated leaves, respectively, compared with control leaves. Treatment of *O. basilicum* leaves with 80 mM NaCl increased the contents of phenolic acids and flavonoid compounds (Table 3).

Table 3. Determination of phenolic acids and flavonoid compounds of basil leaves under salinity stress by HPLC

Compounds (mg/100 g DW)	Treatments			
	C	S1	S2	S3
p-Hydroxybenzoic acid	58.15±0.32	56.2±0.27	48.8±0.33	41.11±0.36
Chlorogenic acid	5.80±0.13	3.23±0.11	7.25±0.15	5.49±0.21
Vanillic acid	10.06±0.16	4.08±0.08	17.94±0.26	3.34±0.18
Caffeic acid	0.83±0.06	nd	0.40±0.02	nd
Syringic acid	0.74±0.02	0.31±0.01	nd	nd
Benzoic acid	498.8±1.25	18.56±0.21	373.37±1.06	30.53±0.42
Ferulic acid	0.87±0.03	2.42±0.05	1.44±0.13	1.21±0.04
Rutin	nd	nd	nd	2.95±0.01
Ellagic	nd	2.02±0.06	nd	1.09±0.02
o- Coumaric acid	40.04±0.71	8.85±0.17	10.10±0.27	3.67±0.12
Resveratrol	nd	nd	nd	78.32±0.35
Cinnamic acid	2.48±0.19	0.40±0.02	nd	nd
Quercetin	86.74±0.41	11.17±0.24	32.22±0.31	14.14±0.24
Rosmarinic acid	41.42±0.50	13.6±0.16	23.69±0.20	26.75±0.39
Myricetin	1406.9±3.56	1326.6±3.74	2394.7±4.19	598.54±2.15
Kaempferol	307.8±1.34	85.3±0.61	85.36±0.77	158.95±1.46
Total	2460.7±4.26	1532.8±2.46	2995.4±4.57	965.97±2.79

- Values are mean± SD of three replicate analyses
- DW: Dry Weight and nd: not detected. C (Control), S1 (40 mM), S2 (80 mM), and S3 (130 mM).

Correlation traits

The correlations recognized among the *in vitro* antioxidant of DPPH and ABTS and the various biochemical responses of untreated and NaCl treated basil leaves are described in Figure 4. In details, the antioxidant capacity (DPPH) was shown to have a strong positive correlation with total flavonoids and *p*-hydroxybenzoic acid contents (0.941 and 0.930) at 1000 µg/mL and (0.793 and 0.917) at 2000 µg/mL, respectively. Furthermore, total flavonoids and *p*-hydroxybenzoic acid levels were found to have a powerful correlation (0.993) and (0.960), respectively, with antioxidant capacity (ABTS) at 3000 µg/mL (Figure 4). On the one side, the amounts of quercetin and benzoic acid were investigated (0.705 and 0.663), respectively at 3000 µg/mL, the content of ferulic acid was displayed a correlation (0.712) with antioxidant capacity (ABTS) at 1000 µg/mL, and moreover, TPC was positively correlated with ABTS scavenging potential (0.659) at 3000 µg/mL, while the DPPH scavenging potential was displayed a low or negative correlation.

Furthermore, the *o*- coumaric acid content was positively correlated (0.635 and 0.534) with antioxidant capacity (DPPH) at 1000 and 2000 µg/mL, respectively, although the correlation was increased from -0.233 to 0.792 with the *o*- coumaric acid content in ABTS scavenging potential. The activity of SOD was strongly correlated with DPPH scavenging (> 0.9) at 1000 and 2000 µg/mL, in spite of the fact that the antioxidant capacity of ABTS gradually ranged from 0.2 to 0.9. Mutually, kaempferol content showed a positive correlation (0.407) with antioxidant capacities at 1000 and 2000 µg/mL for DPPH and ABTS, respectively. On the other hand, the amounts of total soluble sugars and proline showed a positive correlation (0.805 and 0.515, respectively), with antioxidant capacity (only DPPH) at 3000 µg/mL.

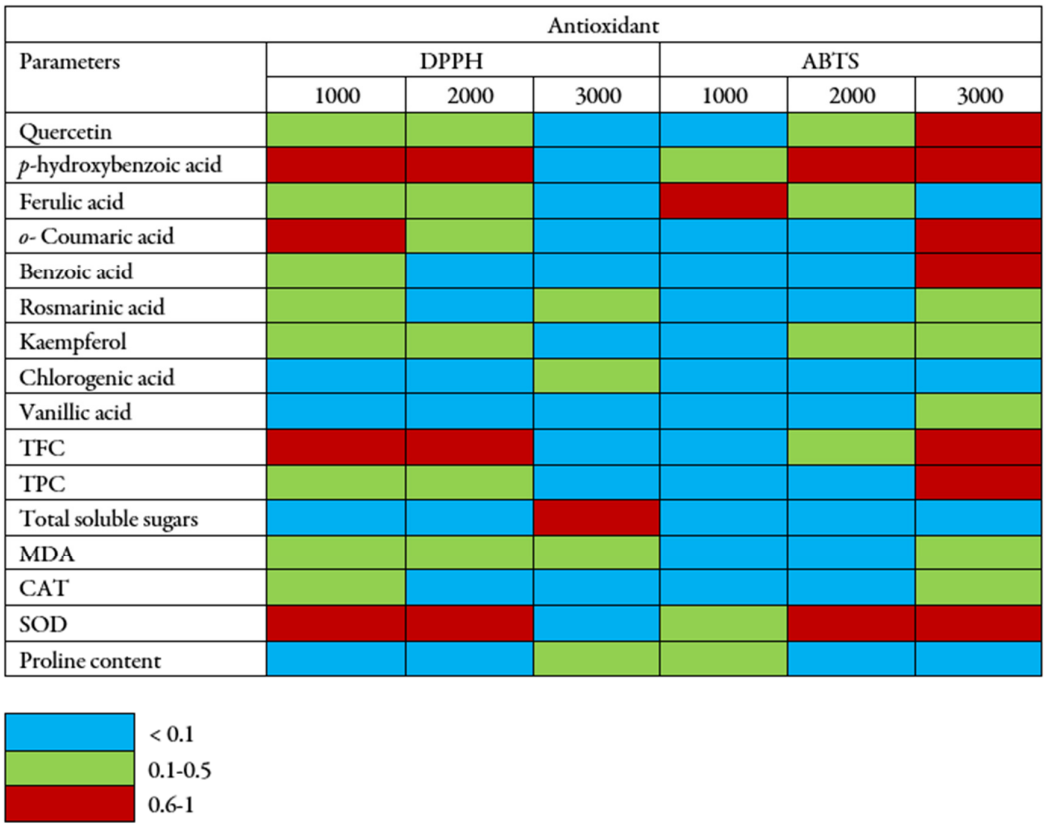


Figure 4. The correlation of antioxidant capacities (ABTS and DPPH) with the various biochemical responses of control and NaCl treated basil leaves
TFC: Total flavonoid content, TPC: Total polyphenol content, MDA: malonaldehyde content, CAT: Catalase, and SOD: Superoxide dismutase.

Discussion

The one of salinity effects is a stomata closure, which causes a decrease of moisture inside leaf tissues (Copolovici *et al*., 2021). Studies have also reported that the degree of salinity tolerance of crops is often inversely associated with its growth rate, since salt tension might directly constrain cell expansion and inhibit water absorption (Munns *et al*., 2006). Moreover, reductions in the biomass, area, and number of *O. basilicum* leaves have been reported in response to salinity stress (Munns and Tester, 2008; Menezes *et al*., 2017). Organic solutes or osmolytes such as sugars, proline, and glycine betaine help plants cope with high salinity stress by adjusting the osmotic pressure of the cytoplasm and maintaining the integrity of photosystem II (Heidari, 2011; Krasensky and Jonak, 2012). Moreover, the organic solutes play critical roles associated with reducing the levels of ROS by forming hydrophilic complexes (Krasensky and Jonak, 2012), scavenging ˙OH radicals, quenching singlet oxygen (Szabados and Savouré, 2010), and activating defensive pathways inside the plant cells (Fariduddin *et al*., 2013)

MDA levels are therefore used to evaluate the extent of membrane lipid peroxidation. The correlation between proline and MDA contents was calculated as a negative correlation (-0.604) (Figure 1 A and B), in agreement with Wang *et al*. (2016) who investigated the converse relationship between proline and MDA contents in stressed -*Arabidopsis thaliana* leaves. The last relationship might be ascribed to the auxiliary roles

of superior proline accumulation at salinity stress through ROS scavenging and the consequent defense of membrane integrity (Wang *et al*., 2016). Also, the increase in the concentration of total soluble sugars in NaCl-treated *O. basilicum* leaves was dose-dependent and significant compared with control leaves (Figure 1, C), this finding disagrees with that reported by Heidari (2011) who found that treatment with 6 ds/m NaCl exhibited no significant effect on carbohydrate content (Heidari, 2011).

Moreover, the concentrations of Na^+ in NaCl-treated *O. basilicum* leaves displayed dose-dependent and significant increase compared with control leaves (Figure 2). Despite the comparatively high levels of Na^+ ion accumulation in *O. basilicum* leaves, the lowest levels of K^+ ions were observed in S1- and S3-treated leaves (Figure 2). Apse and Blumwald (2007) suggested that *O. basilicum* can accumulate and compartmentalize Na^+ ions inside its leaves without undergoing dehydration. Furthermore, the salinity tolerance of *O. basilicum* may be associated with its capacity to (1) sustain K^+ provision to its leaves and (2) maintain Na^+ accumulation in its leaves at levels compatible with normal cellular physiology. Several genes encode transporters responsible for the movement of Na^+ from the xylem of the root to the leaves (Apse and Blumwald, 2007).

Subsequently, to alleviate the ROS mediated oxidative stress, plants utilize an antioxidant defense scheme involving antioxidant enzymes, for instance CAT and SOD, as well as nonenzymatic antioxidants, such as phenolic acids, carotenoids, and flavonoids (Gengmao *et al*., 2015; Wei *et al*., 2015). In similar, the present study clarified the pathways of basil for tolerance the salinity including: elevating the antioxidant enzyme activities including (CAT and SOD) and accumulation of polyphenols and flavonoids (Table 2). The enzyme SOD is obtained in various partitions of the cell and converts the superoxide radicals ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) which removed by peroxidases and catalase. The increase in SOD activity was observed in basil shoots after NaCl treatment, while the decline in SOD activity may be due to extra plant growth; developed plants may improve a suitable enzyme scheme in addition to antioxidant developments.

Furthermore, this finding is in accordance with the data found in the extant study, which revealed that CAT activity was the highest in the S3-treated leaves (Table 2). Generally, the increases or decreases in CAT activity are caused by differences in the period, type, and intensity of stress. A previous study reported that the roots and shoots of *O. basilicum* displayed maximum CAT activity during their early growth phase (i.e., < 14 days) in response to salinity (Sharma *et al*., 2012; Jakovljević *et al*., 2017). The methanolic extracts of the control and salinized *O. basilicum* leaves exhibited higher polyphenol and flavonoid contents in the present study (Table 2) compared with those reported in previous studies. For example, one study reported phenol and flavonoid contents in ethanolic extract of basil leaves were 29.60 mg GAE/g and 19.58 mg QE/g, respectively (Nguyen *et al.,* 2021). This increase was due to the use of methanol solvent that was previously showed a highest efficacy of phenolic compound extraction (Nguyen *et al*., 2020). On the other hand, the decrease in total polyphenol content at 130 mM NaCl was previously referred to inferior capability to adjust reactive oxygen species and the oxidative capacity in *O. basilicum* (Copolovici *et al*., 2021).

Our results showed significant reductions in DPPH scavenging activity in salinized *O. basilicum* leaf extracts (at concentrations of 1000 and 2000 µg/mL) in response to the 130 mM NaCl treatment (Figure 3, A). This finding is reliable with the those of Robatjazi *et al.* (2020) who reported a similar relationship in *O. basilicum* shoots treated with 200 mM NaCl (Robatjazi *et al*., 2020).

The results of the current study correspond with those found in the relevant literature. For instance, Imen *et al*. (2012) observed a decline in the caffeic and rosmarinic acid contents of a fine cultivar of basil following 15 days of Na_2SO_4 stress (Imen *et al*., 2012). Furthermore, the previous studies have also reported a decrease in the *p*-coumaric acid content of wheat leaves in response to salinity , as well as a high abundance of phenolic acids and flavonoid compounds (including vanillic, chlorogenic, ferulic, and ellagic acids and rutin) in the leaves of *Aegilops cylindrical* Host and *Amaranthus tricolor* L. subjected to salinity stress (Kiani *et al*., 2021). As result, differences in the DPPH and ABTS scavenging potentials of control and salinized *O. basilicum* leaves may be due to the presence of kaempferol, myricetin, ferulic acid, and *p*-hydroxybenzoic acid.

The role of phenolic acids and flavonoids in the scavenging of free radicals were showed by Gould and Lister (2006). Generally, the increase in polyphenol contents of treated basil leaves at 80 mM NaCl (Table 2) may be because of their antioxidant properties, which allows them to inhibit lipid peroxidation in response to salinity stress (Sivaci *et al*., 2014).

The total flavonoid, quercetin, and phenolic acids involving (*p*-hydroxybenzoic, benzoic, ferulic, and *o*-coumaric acids) were displayed a positive correlation (Figure 4), in agreement with Isetkomolmat *et al*. (2023) who showed a positive correlation of quercetin with antioxidant capacities of rice husk (Isetkomolmat *et al.,* 2023). As well, the latter study recognized a positive correlation between antioxidant capacity (DPPH) and total flavonoids of safflower (Golkar and Taghizadeh, 2018; El-Beltagi *et al*., 2019) and the positive role of hydroxybenzoic acid of silk in antioxidant activity was previously displayed (Ratha *et al*., 2023). Also, a previous report by Złotek *et al.* (2016) demonstrated a positive relationship between the antioxidant potential and total polyphenol and flavonoid contents of untreated *O. basilicum* leaves, which was validated by our experimental results (Złotek *et al*., 2016). Structurally, the presence of catechol group on ring B of flavonoids and 5-OH group of A ring are explained the positive correlation between antioxidant capacities (DPPH and ABTS) and total flavonoids content (TFC) and some of phenolic acids for basil leaves under NaCl stress. Moreover, the methoxyl group and number of hydroxyl groups in phenolic acids had a determinant role in their antioxidant activity (Moazzen *et al*., 2022). In addition, phenolics mixture showed dose de-pendent activity among concentrations and combinations of radical antioxidants such as phenolics, catechin, *p*-hydroxybenzoic, benzoic, ferulic, coumaric acid, caffeic acid, chrysin, quercetin, kaempferol…etc showed greater activity against TMV infection and their oxidative stress (Dhawi *et al*., 2021). The high contents of antioxidant compounds in these extracts, such as total phenolics and flavonoids, may contribute to their greater total antioxidant capacity. Due to their high redox properties, phenolic compounds are crucial for quenching singlet or triplet oxygen, as well as for the breakdown of peroxides. They also absorb and neutralize free radicals, which helps to prevent oxidative chain reactions (Ramadan *et al*., 2022b; El-Beltagi *et al*., 2022c).

Conclusions

This work suggests the pathway of basil to resist NaCl pressure by showing the correlation of *in vitro* antioxidant potentials with various biochemical responses. In conclusion, the correlation of antioxidant potential with the SOD activity and content of TFC, *o*- coumaric acid, and *p*-hydroxybenzoic acid was powerfully positive. While the CAT activity and the amounts of total soluble sugars, proline, quercetin, TPC, kaempferol, and phenolic acids involved (benzoic, ferulic, chlorogenic, and rosmarinic acids) displayed a moderate correlation with antioxidant potential.

Authors' Contributions

Conceptualization: ASK, MMM, HSEB and HSG; Data curation: ASK, HSEB and HSG; Formal analysis: ASK, HSEB and HSG; Funding acquisition: HSEB; Investigation: MMM, HSEB and HSG; Methodology: ASK and HSG; Project administration: HSEB and ASK; Resources: ASK; Software: ASK and HSEB; Supervision: MMM, HSEB and HSG; Validation: ASK, MMM, HSEB and HSG; Visualization ASK, MMM, HSEB and HSG; Writing - original draft: ASK; Writing - review and editing: ASK, MMM, HSEB and HSG;. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

Authors extend their appreciation to Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (GRANT 3,015).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abbas G, Amjad M, Saqib M, Murtaza B, Asif Naeem M, Shabbir A, Murtaza G (2021). Soil sodicity is more detrimental than salinity for quinoa (*Chenopodium quinoa Willd*.): A multivariate comparison of physiological, biochemical and nutritional quality attributes. Journal of Agronomy and Crop Science 207(1):59-73. *https://doi.org/10.1111/jac.12451*

Aebi H (1984). Catalase *in vitro*. In: Methods in Enzymology. Elsevier, pp 121-126. *https://doi.org/10.1016/S0076-6879(84)05016-3*

Alkuwayti MA, El-Sherif F, Yap Y-K, Khattab S (2020). Foliar application of *Moringa oleifera* leaves extract altered stress-responsive gene expression and enhanced bioactive compounds composition in *Ocimum basilicum*. South African Journal of Botany 129:291-298. *https://doi.org/10.1016/j.sajb.2019.08.001*

Apse MP, Blumwald E (2007). Na^+ transport in plants. FEBS Letters 581:2247-2254. *https://doi.org/10.1016/j.febslet.2007.04.014*

Arunachalam K, Parimelazhagan T (2014). Evaluation of nutritional composition and antioxidant properties of underutilized *Ficus talboti* King fruit for nutraceuticals and food supplements. Journal of Food Science and Technology 51:1260-1268. *https://doi.org/10.1007/s13197-012-0647-6*

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-207. *https://doi.org/10.1007/BF00018060*

Caliskan O, Kurt D, Temizel KE, Odabas MS (2017). Effect of salt stress and irrigation water on growth and development of sweet basil (*Ocimum basilicum* L.). Open Agriculture 2:589-594. *https://doi.org/10.1515/opag-2017-0062*

Christian GD (1970). Atomic-Absorption Spectroscopy; Applications in Griculture, Biology and Medicine (No. 535.84/C933).

Copolovici L, Lupitu A, Moisa C, Taschina, M, Copolovici, DM (2021). The effect of antagonist abiotic stress on bioactive compounds from basil (*Ocimum basilicum*). Applied Sciences 11:9282. *https://doi.org/10.3390/app11199282*

de Azevedo Neto AD, Menezes RV, Gheyi HR, Costa Conceicao Silva P, Cova AMW, Ribas RF, de Oliveira Ribeiro M (2019). Salt-induced changes in solutes, pigments and essential oil of two basil (*Ocimum basilicum* L.) genotypes under hydroponic cultivation. Australian Journal of Crop Science 13:1856-1864. *https://doi.org/10.3316/informit.932511383938802*

Dhawi F, El-Beltagi HS, Abdel-Mobdy YE, Salah SM, Ghaly IS, Abdel-Rahim EA, Mohamed HI, Soliman AM (2021). Synergistic impact of the pomegranate peels and its nanoparticles against the infection of tobacco mosaic virus (TMV). Fresenius Environmental Bulletin 30:731-746.

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.

El-Beltagi H S, Ahmad I, Basit A, Abd El-Lateef HM, Yasir M, Tanveer Shah S, ... Zohaib Ikram M (2022a). Effect of azospirillum and azotobacter species on the performance of cherry tomato under different salinity levels. Gesunde Pflanzen 74(2):487-499. *https://doi.org/10.1007/s10343-022-00625-2*

El-Beltagi HS, Mohamed HI, Abdelazeem AS, Youssef R, Safwat G (2019). GC-MS analysis, antioxidant, antimicrobial and anticancer activities of extracts from *Ficus sycomorus* fruits and leaves. Not. Bot. Horti. Agrobot. Cluj-Napoca 47:493-505. *https://doi.org/10.15835/nbha47211405*

El-Beltagi HS, Mohamed AA, Mohamed HI, Ramadan KM, Barqawi AA, Mansour AT (2022b). Phytochemical and potential properties of seaweeds and their recent applications: A review. Marine Drugs 20(6):342. *https://doi.org/10.3390/md20060342*

El-Beltagi HS, El-Mahdy OM, Mohamed HI, El-Ansary AE (2022c). Antioxidants, antimicrobial, and anticancer activities of purified chitinase of *Talaromyces funiculosus* strain CBS 129594 biosynthesized using Crustacean bio-wastes. Agronomy 12:2818. *https://doi.org/10.3390/agronomy12112818*

Estefan G, Sommer R, Ryan J (2013). Methods of soil, plant, and water analysis. A manual for the West Asia and North Africa region 3:65-119. *https://repo.mel.cgiar.org/handle/20.500.11766/7512*

Fariduddin Q, Varshney P, Yusuf M, Ali A, Ahmad A (2013). Dissecting the role of glycine betaine in plants under abiotic stress. Plant Stress 7:8-18.

Gengmao Z, Yu H, Xing S, Shihui L, Quanmei S, Changhai W (2015). Salinity stress increases secondary metabolites and enzyme activity in safflower. Industrial Crops and Products 64:175-181. *https://doi.org/10.1016/j.indcrop.2014.10.058*

Giannopolitis CN, Ries SK (1977). Superoxide dismutases: I. Occurrence in higher plants. Plant Physiology 59:309-314. *https://doi.org/10.1104/pp.59.2.309*

Golkar P, Taghizadeh M (2018). *In vitro* evaluation of phenolic and osmolite compounds, ionic content, and antioxidant activity in safflower (*Carthamus tinctorius* L.) under salinity stress. Plant Cell, Tissue and Organ Culture (PCTOC) 134:357-368. *https://link.springer.com/article/10.1007/s11240-018-1427-4*

Gould KS, Lister C (2006). Flavonoid functions in plants. Flavonoids: Chemistry, Biochemistry and Applications 397–441. *https://www.cabdirect.org/cabdirect/abstract/20063011833*

Imen T, Cristina S, Baâtour Olfa IR, Mokhtar L, Flavia NI, Zeineb O (2012). Phenolic acids and total antioxidant activity in *Ocimum basilicum L*. grown under Na2SO4 medium. Journal of Medicinal Plants Research 6:5868-5875. *https://doi.org/10.5897/JMPR12.011*

Jabeen N, Ahmad R (2013). The activity of antioxidant enzymes in response to salt stress in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) seedlings raised from seed treated with chitosan. Journal of the Science of Food and Agriculture 93:1699-1705. *https://doi.org/10.1002/jsfa.5953*

Jakovljević DZ, Topuzović MD, Stanković MS, Bojović BM (2017). Changes in antioxidant enzyme activity in response to salinity-induced oxidative stress during early growth of sweet basil. Horticulture, Environment, and Biotechnology 58:240-246. *https://link.springer.com/article/10.1007/s13580-017-0173-6*

Kiani R, Arzani A, Mirmohammady Maibody SAM (2021). Polyphenols, flavonoids, and antioxidant activity involved in salt tolerance in wheat, Aegilops cylindrica and their amphidiploids. Frontiers in Plant Science 12:493. *https://doi.org/10.3389/fpls.2021.646221*

Krasensky J, Jonak C (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Journal of experimental botany 63:1593-1608. *https://doi.org/10.1093/jxb/err460*

Mehdizadeh L, Moghaddam M, Lakzian A (2019). Alleviating negative effects of salinity stress in summer savory (*Satureja hortensis* L.) by biochar application. Acta Physiologiae Plantarum 41:1-13. *https://link.springer.com/article/10.1007/s11738-019-2900-3*

Menezes RV, Azevedo Neto AD, Gheyi HR, Cova AM, Silva HH (2017). Tolerance of basil genotypes to salinity. Journal of Agricultural Science 9:283-295. *https://doi.org/10.5539/jas.v9n11p283*

Moazzen A, Öztinen N, Ak-Sakalli E, Kocsar M (2022). Structure-antiradical activity relationships of 25 natural antioxidant phenolic compounds from different classes. Heliyon 8:e10467. *https://doi.org/10.1016/j.heliyon.2022.e10467*

Mohammed FS, Akgul H, Sevindik M, Khaled BMT (2018). Phenolic content and biological activities of *Rhus coriaria* var. *zebaria*. Fresenius Environmental Bulletin 27(8):5694-5702. *https://www.prt-parlar.de/.../*

Heidari M (2011). Effects of salinity stress on growth, chlorophyll content and osmotic components of two basil (*Ocimum basilicum* L.) genotypes. African Journal of Biotechnology 11:379-384. *https://doi.org/10.5897/ajb11.2572*

Munns R and tester M, (2008). Mechanisms of salinity tolerance annual Review of Plant Biology 59:651-681. *https://doi.org/10.1146/annurev.arplant.59.032607.092911*

Munns R, James RA, Läuchli A (2006). Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany 57:1025-1043. *tps://doi.org/10.1093/jxb/erj100*

Nawaz K, Ashraf M (2010). Exogenous application of glycinebetaine modulates activities of antioxidants in maize plants subjected to salt stress. Journal of Agronomy and Crop Science 196:28-37. *https://doi.org/10.1111/j.1439-037X.2009.00385.x*

Negrao S, Schmöckel SM, Tester M (2017). Evaluating traits contributing to salinity tolerance. Annals of Botany 119:13-26. *https://doi.org/10.1093/aob/mcw253*

Nguyen VT, Nguyen MT, Tran QT, Thinh PV, Bui LM, Le THN, ... Linh HTK (2020). Effect of extraction solvent on total polyphenol content, total flavonoid content, and antioxidant activity of soursop seeds (*Annona muricata* L.). In: IOP Conference Series: Materials Science and Engineering, pp 22063. *https://doi.org/10.1088/1757-899X/736/2/022063/meta*

Nguyen VT, Nguyen NQ, Thi NQN, Thi CQN, Truc TT, Nghi PTB (2021). Studies on chemical, polyphenol content, flavonoid content, and antioxidant activity of sweet basil leaves (*Ocimum basilicum L.*). In: IOP Conference Series: Materials Science and Engineering, pp 12083. *https://doi.org/10.1088/1757-899X/1092/1/012083/meta*

Ordonez AAL, Gomez JD, Vattuone MA (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chemistry 97:452-458. *https://doi.org/10.1016/j.foodchem.2005.05.024*

Osakabe Y, Osakabe K, Shinozaki K, Tran L-SP (2014). Response of plants to water stress. Frontiers in Plant Science 5:86. *https://doi.org/10.3389/fpls.2014.00086*

Prior RL, Wu X, Schaich K (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53:4290-4302. *https://doi.org/10.1021/jf0502698*

Ramadan KMA, El-Beltagi HS, Bendary ESA, Ali HM (2022a). Experimental evaluation of the antioxidant and antitumor activities of thyme and basil essential oils and their phenolic constituents: theoretical antioxidant evaluation. Chemical and Biological Technologies in Agriculture 9:1-11. *https://doi.org/10.1186/s40538-022-00366-4*

Ramadan KMA, El-Beltagi HS, Mohamed HI, Shalaby TA, Galal A, Mansour AT, Aboul Fotouh MM, Bendary ESA (2022b). Antioxidant, anti-cancer activity and phytochemicals profiling of *Kigelia pinnata* fruits. Separations 9:379. *https://doi.org/10.3390/separations9110379*

Ratha J, Yongram C, Panyatip P, Powijitkul P, Siriparu P, Datham S, ... Puthongking P (2023). Polyphenol and tryptophan contents of purple corn (*Zea mays* L.) variety KND and butterfly pea (*Clitoria ternatea*) aqueous extracts: insights into phytochemical profiles with antioxidant activities and PCA analysis. Plants 12:603. *https://doi.org/10.3390/plants12030603*

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26:1231-1237. *https://doi.org/10.1016/S0891-5849(98)00315-3*

Rizvi A, Ahmed B, Khan, MS, El-Beltagi HS, Umar S, Lee J (**2022**). Bioprospecting plant growth promoting rhizobacteria for enhancing the biological properties and phytochemical composition of medicinally important crops. Molecules *27*:1407. *https://doi.org/10.3390/molecules27041407*

Robatjazi R, Roshandel P, Hooshmand S (2020). Benefits of silicon nutrition on growth, physiological and phytochemical attributes of basil upon salinity stress. International Journal of Horticultural Science and Technology 7:37-50. *https://doi.org/10.22059/ijhst.2020.288551.318*

Scagel CF, Lee J, Mitchell JN (2019). Salinity from NaCl changes the nutrient and polyphenolic composition of basil leaves. Industrial Crops and Products 127:119-128. *https://doi.org/10.1016/j.indcrop.2018.10.048*

Schneider CA, Rasband WS, Eliceiri KW (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671-675.

Sevindik M, Akgul H, Pehlivan M, Selamoglu Z (2017). Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. Fresenius Environmental Bulletin 26(7):4757-4763. *https://doi.org/5993f2d7458515c0ce64ce50*

Sharma OP, Bhat TK (2009). DPPH antioxidant assay revisited. Food Chemistry 113:1202-1205. *https://doi.org/10.1016/j.foodchem.2008.08.008*

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2012. *https://doi.org/10.1155/2012/217037*

Sivaci A, Kaya A, Duman S (2014). Effects of ascorbic acid on some physiological changes of pepino (*Solanum muricatum* Ait.) under chilling stress. Acta Biologica Hungarica 65:305-318. *https://doi.org/10.1556/abiol.65.2014.3.7*

Stewart RRC, Bewley JD (1980). Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiology 65:245-248. *https://doi.org/10.1104/pp.65.2.245*

Szabados L, Savouré A (2010). Proline: a multifunctional amino acid. Trends in Plant Science 15:89-97. *https://doi.org/10.1016/j.tplants.2009.11.009*

Talebi M, Moghaddam M, Pirbalouti AG (2018). Methyl jasmonate effects on volatile oil compounds and antioxidant activity of leaf extract of two basil cultivars under salinity stress. Acta Physiologiae Plantarum 40:1-11. *https://doi.org/10.1007/s11738-018-2611-1*

Tarchoune I, Sgherri C, Izzo R, et al (2012). Changes in the antioxidative systems of *Ocimum basilicum* L. (cv. Fine) under different sodium salts. Acta Physiologiae Plantarum 34:1873-1881. *https://doi.org/10.1007/s11738-012-0985-z*

Tester M, Davenport R (2003). Sodium tolerance sodium transport in higher plants. Annals of Botany 91:503-527. *https://doi.org/10.1093/aob/mcg058*

Vlase L, Benedec D, Hanganu D, Damian G, Csillag I, Sevastre B, ... Tilea I (2014). Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum* and *Teucrium chamaedrys*. Molecules 19:5490-5507. *https://doi.org/10.3390/molecules19055490*

Wang F, Zhu H, Chen D, Li Z, Peng R, Yao Q (2016). A grape bHLH transcription factor gene, VvbHLH1, increases the accumulation of flavonoids and enhances salt and drought tolerance in transgenic *Arabidopsis thaliana*. Plant Cell, Tissue and Organ Culture (PCTOC) 125:387-398. *https://doi.org/10.1007/s11240-016-0953-1*

Wei P, Yang Y, Wang F, Chen H (2015). Effects of drought stress on the antioxidant systems in three species of Diospyros L. Horticulture, Environment, and Biotechnology 56:597-605. *https://doi.org/10.1007/s13580-015-0074-5*

Wisetkomolmat J, Arjin C, Hongsibsong S, Ruksiriwanich W, Niwat C, Tiyayon P, ... Sringarm K (2023). Antioxidant activities and characterization of polyphenols from selected northern Thai Rice Husks: Relation with seed attributes. Rice Science 30:148-159. *https://doi.org/10.1016/j.rsci.2023.01.007*

Zabka M, Pavela R, Prokinova E (2014). Antifungal activity and chemical composition of twenty essential oils against significant indoor and outdoor toxigenic and aeroallergenic fungi. Chemosphere 112:443-448. *https://doi.org/10.1016/j.chemosphere.2014.05.014*

Złotek U, Szymanowska U, Karaś M, Świeca M (2016). Antioxidative and anti-inflammatory potential of phenolics from purple basil (*Ocimum basilicum L.*) leaves induced by jasmonic, arachidonic and $β$-aminobutyric acid elicitation. International Journal of Food Science & Technology 51:163-170. *https://doi.org/10.1111/ijfs.12970*