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EFFECT OF NaCl SALINITY ON BIOACTIVE COMPOUND ACCUMULATION AND ANTIOXIDANT POTENTIAL IN BASIL (*OCIMUM BASILICUM* L.): IMPLICATIONS FOR NUTRITIONAL VALUE

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Abstract: Salt stress is one of the most prevalent abiotic stresses limiting plant productivity worldwide, yet moderate salinity can act as an elicitor of secondary metabolism. This study investigated the effect of NaCl concentrations on germination, vegetative growth, photosynthetic pigment content, total polyphenol content (TPC), and ABTS (3-ethylbenzothiazoline-6-sulfonic acid) radical-scavenging capacity in basil (*Ocimum basilicum* L.). Following assessment of data normality and homogeneity of variance, the nonparametric Kruskal-Wallis test was applied to leaf number, while one-way ANOVA with Tukey HSD post hoc testing was applied to TPC and ABTS data. Low salinity (V1, 17.1mM NaCl) stimulated fresh biomass. Leaf number was significantly reduced in V2 and V3 relative to the control. Chlorophyll a and total chlorophyll were highest in V3. TPC decreased progressively with salinity, with V0 and V1 significantly higher than V3. ABTS antioxidant capacity showed a contrasting non-linear pattern, being significantly higher in V3 than in V0, V1, and V2. Pearson correlation across all triplicates ($n = 12$) between TPC and ABTS was significantly negative, indicating that non-phenolic compounds likely sustain antioxidant capacity in salt-stressed basil. The results provide a basis for optimizing controlled cultivation of basil to enhance its bioactive profile for functional food applications.

Keywords: *Ocimum basilicum*, salt stress, NaCl, photosynthetic pigments, polyphenols, ABTS, antioxidant capacity, functional food

1. Introduction

Basil (*Ocimum basilicum* L., Lamiaceae) is one of the most widely cultivated culinary and medicinal herbs globally, prized for its essential oil profile, aromatic properties, and rich content of bioactive phytochemicals, including phenolic acids, flavonoids, and terpenoids (Zheljazkov et al., 2008; Ekren et al., 2012). Beyond its culinary use, basil is increasingly studied for its antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activities, positioning it as a candidate crop for functional food development (Kwee and Niemeyer, 2011).

The phenolic profile of basil varies considerably among cultivars and accessions, reflecting differences in the relative content of these major phenolic acids and in the resulting antioxidant activity (Kwee and Niemeyer, 2011). Beyond their role as natural antioxidants that scavenge free radicals and limit oxidative stress, basil phenolics and other phytonutrients have been associated with a broad range of beneficial health effects, including antimicrobial, anti-inflammatory, antidiabetic, and cardioprotective properties (Filip 2017). These characteristics make basil an important model species for investigating how phenolic content and antioxidant capacity are influenced by growing and cultivation conditions, as addressed in the present study.

Soil salinity is one of the most damaging abiotic stresses limiting agricultural production, affecting over 800 million hectares of arable land worldwide (FAO, 2021). Sodium chloride (NaCl) induces osmotic stress, ionic toxicity, and oxidative damage in plants, leading to impaired germination, reduced growth, and altered metabolism (Munns and Tester, 2008). However, an emerging body of evidence suggests that mild-to-moderate salt stress can function as an abiotic elicitor of secondary metabolite biosynthesis in certain plant species, potentially increasing the nutraceutical value of crops grown under controlled conditions (Rouphael et al., 2018; Chrysargyris et al., 2019).

In basil, the relationship between salinity and secondary metabolism is complex and concentration-dependent. Low NaCl concentrations have been reported to stimulate phenolic compound accumulation, while high concentrations generally impair photosynthetic efficiency and biomass accumulation (Tarchoune et al., 2010; Attia et al., 2021). The modulation of photosynthetic pigments — chlorophylls and carotenoids — under salt stress reflects the interplay between oxidative damage and antioxidant protective responses (Ashraf and Harris, 2013). Despite growing interest in salinity-driven enhancement of bioactive compounds, the dose-response relationship and its nutritional implications remain incompletely characterized, particularly under controlled greenhouse conditions that simulate realistic urban or indoor farming scenarios.

The present study evaluated the effect of four NaCl concentrations on germination parameters, vegetative growth, photosynthetic pigments, total phenolic content, and ABTS radical-scavenging antioxidant capacity in basil seedlings grown in a controlled greenhouse environment. The aim was to identify the salinity level that optimizes bioactive compound accumulation without unacceptably compromising plant growth, with implications for controlled-environment cultivation strategies aimed at producing high-value functional ingredients.

Building on existing evidence that abiotic stress can act as an elicitor of secondary metabolism in medicinal and aromatic plants, we hypothesized that moderate NaCl exposure would stimulate the synthesis of phenolic compounds and enhance the antioxidant capacity of basil without significantly compromising vegetative growth, whereas prolonged exposure to high NaCl concentrations would reduce photosynthetic pigment content and biomass accumulation as a consequence of osmotic stress. This working hypothesis was addressed through two specific research questions: (i) Does low-to-moderate salinity enhance phenolic content and antioxidant capacity relative to non-saline control conditions without compromising plant growth and biomass accumulation? (ii) Does high salinity reduce photosynthetic pigment content and biomass yield as a consequence of osmotic and oxidative stress?

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment was conducted at the greenhouse facilities of the Botanical Garden of the University of Medicine, Pharmacy, Sciences and Technology "George Emil Palade" of Târgu Mureș, Romania. Seeds of *Ocimum basilicum* L., from the collection of the Botanical Garden, were sown in plastic pots (diameter 10–12 cm) filled with commercial peat substrate. Three seeds were planted per pot, and 120 pots were used, distributed equally among four treatment groups (30 pots per treatment). Growth conditions were maintained at 25 ± 2 °C, with a relative humidity of 50–60% and a photoperiod of 12 h light/12 h dark, supplemented with artificial lighting when natural irradiance was insufficient. The chemical composition and electrical conductivity of the commercial peat substrate were not characterized prior to the experiment.

2.2. Salinity Treatments

Four salinity levels were applied via irrigation with NaCl solutions prepared in distilled water (**Table 1**). Each pot received 30 mL of the corresponding treatment solution at planting, at 7 days post-planting, and at 10 days post-planting, followed by regular watering with the treatment solution combined with plain water to maintain substrate moisture.

Salt tolerance varies widely across plant species; for many non-halophytic species, salinity stress is generally classified as low at around 50 mM NaCl, moderate at around 100 mM NaCl, and severe at 150–200 mM NaCl (Munns and Tester, 2008). However, basil (*Ocimum basilicum* L.) is documented as a moderately salt-tolerant species owing to its capacity to exclude Na^+ ions at the root level (Ciriello et al., 2024), and basil-specific dose-response studies have consequently established somewhat higher numerical thresholds for these categories. Lazarević et al. (2021) classified 100 mM NaCl as a moderate and 200 mM NaCl as a severe salinity stress in basil, while Khaliq et al. (2017) reported that basil tolerates salinity up to approximately 150 mM NaCl before photosynthetic performance becomes severely compromised. At the lower end of the range, Mousa et al. (2020) showed that ~1,000 ppm NaCl (~17 mM, numerically close to our V1 treatment) stimulated growth parameters in basil cv. 'Genovese' relative to untreated controls, whereas 2,000–4,000 ppm NaCl (~34–68 mM) progressively reduced growth — supporting the classification of our V1 concentration (17.1 mM) as a low, sub-inhibitory salinity level. Our V2 treatment (51.3 mM) falls within the range where basil studies report measurable but moderate growth without growth arrest (Menezes et al., 2017; Mousa et al., 2020), while our V3 treatment (171.1 mM) lies within the high/severe stress range established specifically for basil (Lazarević et al., 2021; Khaliq et al., 2017).

Table 1. NaCl salinity treatments applied to *Ocimum basilicum* L.

Treatment	NaCl concentration (mM)	NaCl per 500 mL (g)	Salinity level
V0	0	0	Control
V1	17,1	0.5	Low
V2	51,3	1.5	Moderate
V3	171,1	5.0	High

2.3. Germination Assessment

Germination capacity (GC) was recorded at 35 days post-sowing as the final cumulative germination count. This index was expressed as a percentage of the total number of seeds sown (3 seeds per pot).

2.4. Growth Parameters

At harvest, leaves were separated from stems and weighed individually using a KERN ACJ300-4M analytical balance (KERN & Sohn GmbH, Balingen, Germany). Leaf fresh weight (FW) and stem FW were recorded per plant. Leaf number per plant was counted.

2.5. Preparation of Plant Extracts

Ultrasound-assisted extraction was performed using a Hielscher UP200St processor (Hielscher Ultrasonics GmbH, Teltow, Germany) at 55% amplitude for 15 min with 40% ethanol at a solid-to-solvent ratio of 1:20 (1 g in 20 mL). Extracts were centrifuged at 1790 × g (4000 rpm; Nahita Blue 2615/1 digital centrifuge, Auxilab S.L., Beriain, Spain) for 10 minutes. For antioxidant and phenolic compound analyses, plant material was lyophilized using a Biobase BK-FD125S freeze dryer (Biobase Co. Ltd., Jinan, China).

2.6. Determination of Photosynthetic Pigments

For dry weight determination, a separate portion of the leaf samples was subjected to natural dehydration. Fresh leaves were spread in a single layer on paper and maintained at room temperature in a dark, well-ventilated environment protected from direct light exposure.

For photosynthetic pigment determination, 0.05 g dry leaf material was homogenized in 10 mL of 80% acetone - a mass selected to remain within the linear absorbance range of the spectrophotometer for this matrix and solvent system; the filtered extract was kept in aluminum-foil-covered tubes at −20°C until spectrophotometric analysis. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids were determined spectrophotometrically on dry leaves using an Analytik Jena SPECORD 200 PLUS spectrophotometer (Analytik Jena AG, Jena, Germany). Absorbances were recorded at 663, 646, and 470 nm. Pigment concentrations were calculated according to the equations of Lichtenthaler and Wellburn (1983): $\text{Chl a} = 12.21 \times A_{663} - 2.81 \times A_{646}$; $\text{Chl b} = 20.13 \times A_{646} - 5.03 \times A_{663}$; $\text{Carotenoids} = (1000 \times A_{470} - 3.27 \times \text{Chl a} - 104 \times \text{Chl b})/229$ (all expressed as mg g^{-1} DW after dividing by sample mass and adjusting for extraction volume). Measurements were performed in triplicate.

2.7. Determination of Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu colorimetric method (Delgado-Alvarado et al., 2022) adapted for microplate format using a Thermo Scientific Multiskan FC microplate reader (Thermo Fisher Scientific, Singapore). In a 96-well microplate, 20 µL of each lyophilised extract (measured in triplicate) was mixed with 100 µL of 10-fold diluted Folin-Ciocalteu reagent (Merck KGaA, Darmstadt, Germany) and incubated in the dark for 3 min. Subsequently, 80 µL of 7.5% Na_2CO_3 solution was added, and the mixture was incubated in the dark for 30 min. Absorbance was read at 765 nm. Results were expressed as milligrams of gallic acid equivalent per milliliter of extract (mg GAE mL^{-1}) using a gallic acid calibration curve: $y = 3.0236x + 0.055$, $R^2 = 0.9979$.

2.8. Determination of Antioxidant Capacity (ABTS Assay)

The radical-scavenging activity of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was assessed using the $ABTS^+$

decolorization method (Tanase et al. 2022). The radical cation was generated by reacting 25 mg ABTS dissolved in 25 mL distilled water with 9.375 mg $K_2S_2O_8$ (≥99%, Carl Roth GmbH, Karlsruhe, Germany) dissolved in 25 mL distilled water (1:1 ratio), followed by 14 h incubation in the dark at room temperature. The working $ABTS^+$ solution was diluted to an absorbance of ~0.70 at 734 nm. Lyophilized extracts (1.5 mg in 1.5 mL distilled water) were measured in triplicate. In a 96-well microplate, 20 µL of extract (or distilled water as the blank) was mixed with 200 µL of $ABTS^+$ solution and incubated for 6 min in the dark at room temperature. Absorbance was recorded at 734 nm. Results were expressed as mg Trolox equivalents per mL extract (mg TE mL^{-1}) using a Trolox calibration curve (Sigma-Aldrich, St. Louis, MO, USA): $y = 0.297x + 0.8459$, $R^2 = 0.9992$.

2.9. Statistical Analysis

All data are expressed as mean ± standard deviation (SD) from three replicates, except leaf number, which was recorded for each individual plant and is additionally reported as the median and interquartile range (IQR). Normality of data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test, prior to selecting parametric or non-parametric procedures. For TPC and ABTS data, both assumptions were satisfied (Shapiro-Wilk, $p > 0.05$ for all groups; Levene's test, $p = 0.518$ and $p = 0.690$, respectively), justifying the use of one-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc test ($\alpha = 0.05$). For leaf number, the data significantly deviated from a normal distribution (Shapiro-Wilk test on pooled residuals, $p = 0.0003$), despite homogeneous variances across groups (Levene's test, $p = 0.954$); therefore, the non-parametric Kruskal-Wallis test was applied, followed by pairwise Mann-Whitney U tests with Bonferroni correction for multiple comparisons. Pearson correlation analysis was performed between TPC and ABTS values across all triplicates (n=12). For photosynthetic pigment data, only mean absorbance values from analytical triplicates were available at the treatment level; descriptive statistics and trends are reported without formal hypothesis testing. Statistical analyses were conducted in Python (SciPy library v.1.13). Significance thresholds: $^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

3. Results

3.1. Germination Parameters

Despite The effect of NaCl on seed germination is illustrated in **Figure 1.** At this early 7-day checkpoint — well before the 35-day final assessment — V3 (171.1 mM NaCl) had no visible germinated seedlings, whereas V0 showed germination in 6 of 30 pots monitored; this early observation reflects only the proportion of seeds that had emerged within the first week and does not indicate poor overall germination capacity, which reached 70.0% in V0 by the final 35-day assessment. Germination capacity, recorded at 35 days post-sowing, was highest in V0 (70.0%) and V2 (73.3%), with V1 (60.0%) and V3 (63.3%) showing somewhat lower values (**Figure 1**).

3.2. Vegetative Growth Parameters

Fresh biomass data at harvest are illustrated in **Figure 2**. Plants treated with low salinity (V1, 17.1 mM NaCl) showed the highest mean leaf fresh weight (8.73 g $plant^{-1}$) and stem fresh weight (3.81 g $plant^{-1}$), exceeding control values (6.77 and 2.61 g $plant^{-1}$, respectively) by approximately 29% and 46%, respectively.

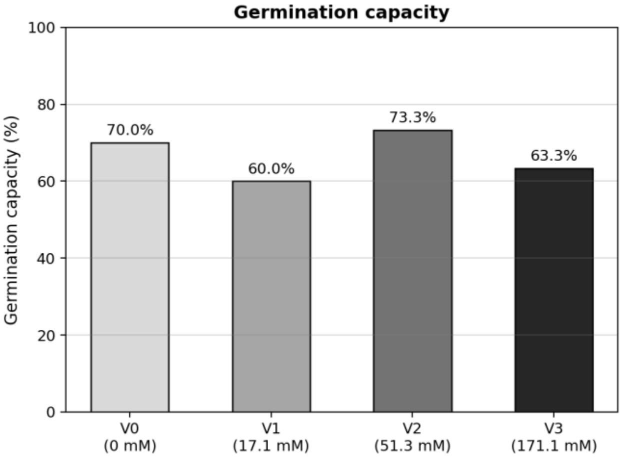

Fig. 1. Germination capacity (%) of *Ocimum basilicum* L. seeds under NaCl treatments (mean of 30 seeds per treatment). V0 = 0 mM; V1 = 17.1 mM; V2 = 51.3 mM; V3 = 171.1 mM NaCl.

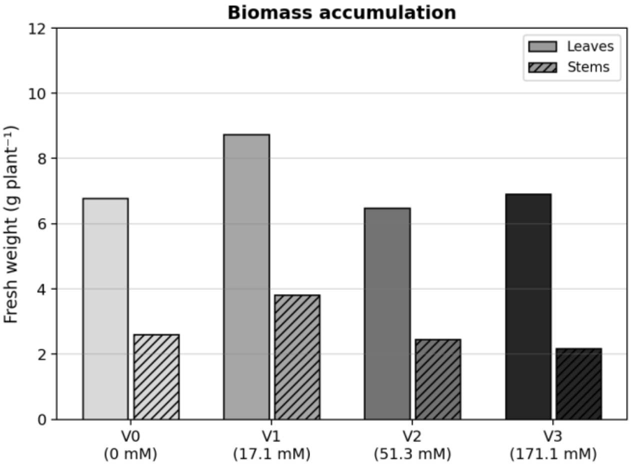

Fig. 2. Biomass accumulation (leaf and stem fresh weight, g plant^{-1}) *in Ocimum basilicum* L. under NaCl treatments V0 = 0 mM; V1 = 17.1 mM; V2 = 51.3 mM; V3 = 171.1 mM NaCl.

Moderate salinity (V2, 51.3 mM) produced leaf and stem fresh weights of 6.48 and 2.46 g plant^{-1}, while high salinity (V3, 171.1 mM) produced 6.91 and 2.18 g plant^{-1}, respectively — both yielding total shoot fresh weights (8.94 and 9.09 g plant^{-1}) comparable to the control (9.38 g plant^{-1}). Fresh weight parameters were evaluated descriptively; no formal statistical hypothesis testing was applied to biomass data, in contrast to leaf number (below), for which individual-plant replication permitted formal testing.

As leaf number data significantly deviated from a normal distribution, the non-parametric Kruskal-Wallis test was applied to individual-plant leaf counts (V0: median 7.0, IQR 6.0–7.2, n = 20; V1: median 6.5, IQR 6.0–7.0, n = 16; V2: median 5.5, IQR 5.0–6.0, n = 24; V3: median 6.0, IQR 4.8–6.0, n = 20), revealing a statistically significant treatment effect (H = 15.041, p = 0.0018). Pairwise Mann-Whitney U tests with Bonferroni correction showed that V2 and V3 had significantly fewer leaves than V0 (p_adj = 0.013 and p_adj = 0.026, respectively), while V1 did not differ significantly from V0 (p_adj = 1.000); V2 and V3 were not significantly different from each other (p_adj = 1.000), and V1 did not differ significantly from V2 or V3 after correction (p_adj = 0.077 and p_adj = 0.137, respectively) (**Figure 3**).

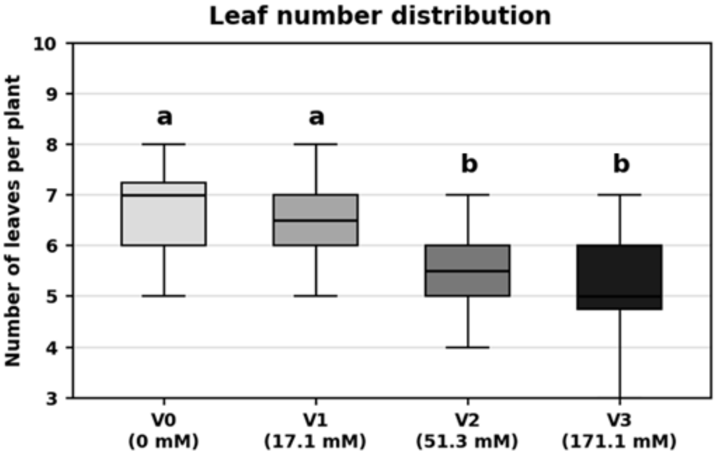

Fig. 3. Leaf number per plant in *Ocimum basilicum* L. under NaCl treatments (boxplots; different letters denote significant differences, Mann-Whitney U with Bonferroni correction, p_adj < 0.05). V0 = 0 mM; V1 = 17.1 mM; V2 = 51.3 mM; V3 = 171.1 mM NaCl.

3.3. Photosynthetic Pigments

Pigment concentrations from dry leaves are illustrated in **Figure 4**. Chlorophyll a ranged from 7.20 mg g^{-1} DW (V2) to 8.58 mg g^{-1} DW (V3), with V3 exceeding the control by ~17.5%. Total chlorophyll (Chl a + b) was also highest in V3 (13.15 mg g^{-1} DW). The Chl a/b ratio was highest in V3 (1.88) and lowest in V1 (1.69). Carotenoid content was slightly reduced in V1 (1.39 mg g^{-1} DW) relative to the control (1.66 mg g^{-1} DW) and marginally elevated in V3 (1.70 mg g^{-1} DW).

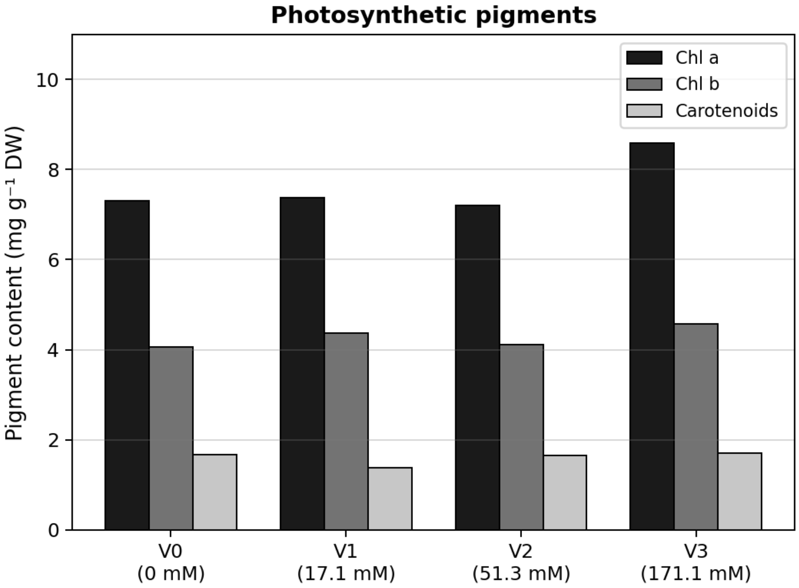

Fig. 4. Photosynthetic pigment content (mg g^{-1} DW) in *Ocimum basilicum* L. under NaCl treatments (means of analytical triplicates). V0 = 0 mM; V1 = 17.1 mM; V2 = 51.3 mM; V3 = 171.1 mM NaCl.

3.4. Total Polyphenol Content (TPC)

TPC values determined from three independent biological replicates per treatment are shown in **Figure 5**. A statistically significant treatment effect was found (one-way ANOVA: F = 7.936, p = 0.0088). TPC decreased progressively with increasing NaCl concentration: V0 (0.123 ± 0.019 mg GAE mL^{-1}), V1 (0.110 ± 0.015), V2 (0.104 ± 0.012), and V3 (0.072 ± 0.003). Tukey HSD post-hoc analysis revealed that V0 was significantly higher than V3 (p = 0.0069), and V1 was significantly higher than V3 (p = 0.0325). No significant differences were detected between V0, V1, and V2, nor between V2 and V3 ($p > 0.05$). This indicates that high salinity (171.1 mM) substantially suppressed phenolic compound biosynthesis or stability, reducing TPC by approximately 41.5% relative to the control.

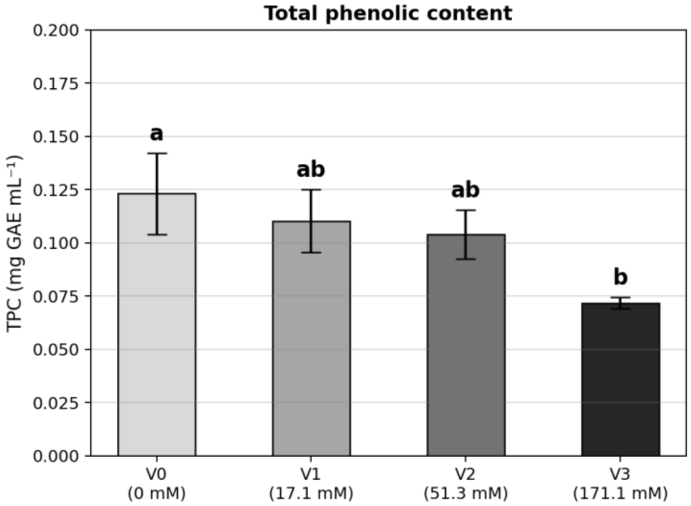

Fig. 5. Total phenolic content (TPC, mg GAE mL^{-1} ± SD) of *Ocimum basilicum* L. extracts under NaCl treatments (different letters denote significant differences, Tukey HSD, $p < 0.05$). V0 = 0 mM; V1 = 17.1 mM; V2 = 51.3 mM; V3 = 171.1 mM NaCl.

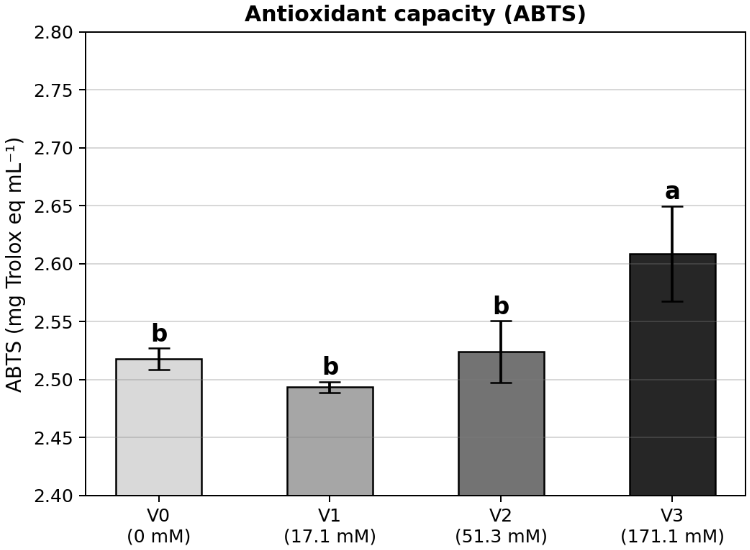

Fig. 6. ABTS antioxidant capacity (mg Trolox eq mL^{-1} ± SD) of *Ocimum basilicum* L. extracts under NaCl treatments (different letters denote significant differences, Tukey HSD, $p < 0.05$). V0 = 0 mM; V1 = 17.1 mM; V2 = 51.3 mM; V3 = 171.1 mM NaCl.

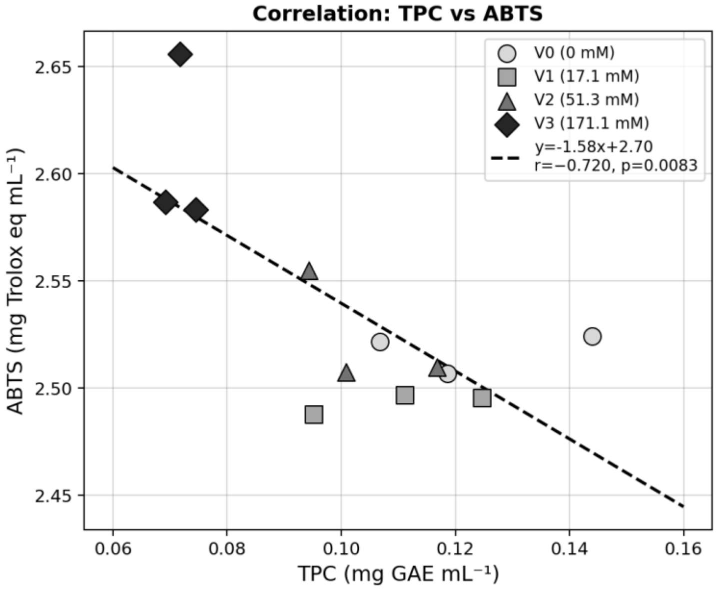

Fig. 7. Pearson correlation between total phenolic content (TPC, mg GAE mL^{-1}) and ABTS radical-scavenging capacity (mg Trolox eq mL^{-1}) across all biological replicates (n = 12). r = −0.720, p = 0.0083

3.5. Antioxidant Capacity (ABTS)

ABTS radical-scavenging capacity (**Figure 6**) showed a significant treatment effect (one-way ANOVA: F = 12.065, p = 0.0024). Values were: V0 (2.518 ± 0.009), V1 (2.493 ± 0.005), V2 (2.524 ± 0.027), and V3 (2.609 ± 0.041 mg TE mL^{-1}). Tukey HSD analysis revealed that V3 was significantly higher than V0 (p = 0.0092), V1 (p = 0.0022), and V2 (p = 0.0138), while V0, V1, and V2 did not differ significantly from each other ($p > 0.05$). The ~4.6% increase in ABTS at V3 relative to the control, despite the lowest TPC, suggests activation of non-phenolic antioxidant pathways under high-salt stress.

3.6. Correlation between TPC and Antioxidant Capacity

Pearson correlation analysis between TPC and ABTS across all 12 biological replicates (3 per treatment × 4 treatments) yielded r = −0.720 (p = 0.0083) (**Figure 7**). This statistically significant negative correlation indicates that treatments and replicates with higher phenolic content were systematically associated with lower ABTS radical-scavenging activity, and vice versa. When computed using only treatment means (n = 4), the trend was similar (r = −0.899, p = 0.101), though non-significant due to the low statistical power at that level. The significant result at the replicate level (n = 12) provides stronger evidence for a genuine decoupling between phenolic compound accumulation and ABTS-measured antioxidant capacity in basil under NaCl stress.

4. Discussions

The present study examined the dose-dependent effects of NaCl salinity on germination, vegetative growth, photosynthetic pigments, phenolic content, and antioxidant capacity in basil under controlled greenhouse conditions. Statistical analysis with biological replicates (n = 3 per treatment) for TPC and ABTS, complemented by individual-plant data for growth parameters, enabled rigorous hypothesis testing and several important biological insights.

No clear monotonic inhibitory trend was observed in germination parameters. The moderate-salinity treatment V2 (51.3 mM) showed the highest germination capacity (73.3%), surpassing the control. This is consistent with reports that mild osmotic priming can stimulate germination in some species by accelerating water uptake and activating germination-associated enzymes (Farhoudi et al., 2011). The delayed germination observed at the highest salt concentration (V3) during the first week — with no visible seedlings — followed by recovery to 63.3% final germination capacity, suggests initial osmotic inhibition overcome by adaptive mechanisms in subsequent weeks.

The stimulatory effect of low salinity (V1, 17.1 mM NaCl) on biomass — with leaf and stem FW both exceeding control values by ~29% and ~46%, respectively — is consistent with a hormetic response, whereby a subinhibitory stressor stimulates growth. This phenomenon has been described in basil and other Lamiaceae under mild NaCl stress (Bernstein et al., 2010; Tarchoune et al., 2012). The significant reduction in leaf number observed at V2 and V3 reflects a concentration-dependent suppression of leaf initiation or accelerated senescence above 51.3 mM NaCl.

The paradoxical increase in total chlorophyll at V3 relative to control — despite the highest ionic stress — may reflect osmotic adjustment through chloroplast reorganization, or a leaf-age effect if V3 plants retained fewer but more mature, pigment-dense leaves at harvest. Similar non-linear chlorophyll responses under salt stress have been reported in basil (Attia et al., 2021) and other glycophytic herbs (Ashraf and Harris, 2013). The slight decrease in carotenoids at V1 relative to control is less straightforward and may reflect developmental variation in this small sample, given that carotenoids typically accumulate under stress as photoprotective pigments.

The statistically significant, progressive decrease in TPC with increasing NaCl contrasts with several published reports suggesting that moderate salt stress stimulates phenolic biosynthesis in basil (Tarchoune et al., 2010; Chrysargyris et al., 2019). The NaCl concentrations used here (17.1–171.1 mM) may have exceeded the mild-stress optimum for phenolic elicitation in this cultivar, with V3 representing clearly inhibitory ionic levels. The 41.5% reduction in TPC at V3 is consistent with oxidative damage to phenolic biosynthetic enzymes and metabolic disruption expected under severe salt stress. Importantly, the use of three independent biological replicates in this study allows this conclusion to be made with statistical confidence, strengthening the validity of the finding compared with single-pool extraction approaches.

The most striking finding of this study is the significant increase in ABTS antioxidant capacity at V3, despite V3 having the lowest TPC. This decoupling is supported by the statistically significant negative Pearson correlation between TPC and ABTS across all replicates. This pattern strongly suggests that non-phenolic antioxidants are the primary contributors to radical-scavenging activity under high salinity. Candidates include ascorbate, tocopherols, and stress-induced terpenoids such as rosmarinic acid precursors or volatile essential oil components known to accumulate in basil under stress conditions (Munns and Tester, 2008; Rouphael et al., 2018). The ABTS assay measures total hydrogen-donating and electron-transfer capacity regardless of compound class, and is therefore sensitive to these alternative antioxidant pools. These findings caution against using TPC alone as a proxy for antioxidant capacity in stress physiology studies, particularly when comparing treatments with divergent metabolic profiles.

From an applied perspective, V1 (17.1 mM NaCl) emerges as the most favorable treatment for controlled basil cultivation, maximizing biomass yield without reducing polyphenol content. For applications where enhanced ABTS antioxidant capacity is the target — at the cost of lower phenolic content and reduced leaf number — V3 (171.1 mM) may be relevant, though the practical stress imposed on plants and the reduced harvestable biomass limit its utility for most commercial scenarios.

Conclusions

This study demonstrates that NaCl salinity exerts concentration-dependent effects on vegetative growth, and statistically significant effects on leaf number, total phenolic content and antioxidant capacity in *Ocimum basilicum*. Low salinity (17.1 mM) stimulated fresh biomass accumulation (hormetic response) without significantly reducing leaf number, while moderate and high salinity (51.3 – 171.1 mM) significantly suppressed leaf production. Chlorophyll content increased at 171.1 mM NaCl, consistent with a stress-adaptive pigment response. TPC decreased progressively with salinity, with V3 significantly lower than V0 and V1, whereas ABTS antioxidant capacity was significantly elevated in V3. The significant negative Pearson correlation between TPC and ABTS indicates that antioxidant activity under high salinity is driven by non-phenolic compounds. These findings have practical implications for optimizing basil cultivation under controlled conditions, with low salinity identified as the optimal strategy for maximizing both biomass and phenolic yields. Future work should include flavonoid profiling, DPPH and FRAP assays, essential oil quantification by GC-MS, and expanded biological replication for pigment determinations.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conceptualization: Conceptualization: C.T.; Data curation: S.S.; Formal analysis: S.S., I.N. and C.T.; Investigation: C.T., I.N. and S.S.; Methodology: S.S., I.N., M.G. and C.T.; Project administration: C.T.; Resources: C.T.; Supervision: C.T.; Validation: I.N. and C.T.; Visualization: C.T.; Writing – original draft: S.S., Preparation: S.S.; Writing – review & editing: I.N. and C.T.

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During the preparation of this manuscript, the authors used Claude to assist with language editing and the generation of data visualizations. The authors reviewed and edited all AI-assisted output and take full responsibility for the accuracy and integrity of the content presented in this publication.

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