AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
**ISSN: 2997-7185**

Article

# Effect of Salt Stress Levels and Spray Application of Salicylic Acid on The Quantitative Content of Phenolic Compounds and Flavonoids in Basil Leaves (*Ocimum basilicum* L) by HPLC

**Hiba Jomaa Al-Fahdawi[1], Ghada Saad Salih[2]**
1. Field Crops Department, College of Agriculture, University of Tikrit, Tikrit, Iraq
2. Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq
Correspondance: Hiba2020@tu.edu.iq, gh.s.s003@tu.edu.iq

**Abstract:** Using high-performance liquid chromatography (HPLC), the research experiment was conducted at the College of Agriculture Research Station at Tikrit University during the 2024–2025 growing season to examine the effects of different salt stress levels and the subsequent foliar spraying of salicylic acid (SA) on the quantitative content of phenolic compounds and flavonoids in the leaves of sweet basil (Ocimum basilicum L). Four salt stress levels (0, 4, 8, and 12 dS/m2) and three salicylic acid concentrations (0, 1, and 2 mmol) with three repetitions were used in a randomized complete block design (RCBD). The findings demonstrated that vegetative development markers, including dormant height, number of branches, and leaf weight, significantly decreased under increased salt stress, reaching up to 68.9% at the maximum salinity level. On the other hand, as a defense mechanism, the leaves' concentration of flavonoid and phenolic chemicals rose. Rosmarinic acid, cicuric acid, chlorogenic acid, caffeic acid, quercetin, and rutin accumulation were all improved by salicylic acid at a dosage of 1 mmol, which also considerably lessened the adverse effects of salt. The greatest concentrations of total phenolic compounds, 4924.7 mg/kg dry weight, were obtained when salinity of 8 dS/m² and salicylic acid at 1 mmol interacted.

**Keywords:** Flavonoids, Phenolic Compounds, Salicylic Acid, Sweet Basil, HPLC

**Citation:** Al-Fahdawi, H. J., Salih, G. S. Effect of Salt Stress Levels and Spray Application of Salicylic Acid on The Quantitative Content of Phenolic Compounds and Flavonoids in Basil Leaves (*Ocimum basilicum* L) by HPLC. American Journal of Biology and Natural Sciences 2026, 3(6), 64-71.

Received: 20th Apr 2026
Revised: 05th May 2026
Accepted: 25th May 2026
Published: 10th Jun 2026

## Introduction

Holy basil (*Ocimum sanctum L.*), a medicinal plant belonging to the Lamiaceae family, has long been recognized for its therapeutic importance. Traditionally, it has been widely utilized for the prevention and treatment of various diseases [1]. Basil is considered a valuable source of vitamins and contains considerable amounts of bioactive compounds, including eugenol, methyl eugenol, phenolic compounds, flavonoids, terpenoids, neolignans, and fatty acid derivatives. These phytochemical constituents contribute to its diverse pharmacological activities, influencing multiple biological functions through antioxidant, anticancer, antiasthmatic, anti-inflammatory, antiallergic, antidiabetic, and stress-relieving properties [2].

Stress is any stimulation that upsets a plant's metabolic equilibrium, which causes significant alterations in the plant's growth and development. Soil salinity is a significant global concern that limits agricultural productivity [3]. About 20% of the global irrigated agricultural area is impacted by salinity, a situation exacerbated by natural contamination, inadequate irrigation methods, and climate change. Salinity stress is a prominent abiotic element that adversely affects agricultural productivity by diminishing photosynthetic capacity through its impact on stomatal osmotic pressure and partitioning affinity [4]. The influence of salt stress on plants is contingent upon the kind and intensity of the salt, the duration of exposure, the salt tolerance of the plant genotype, and the developmental stage.

Elevated sodium levels impose both ionic and osmotic stress on plants by restricting the absorption of water and essential mineral nutrients from the soil. Salinity stress induces numerous physiological and molecular disturbances that adversely affect plant development, primarily through the suppression of photosynthetic activity and the inhibition of cell division and expansion processes [5]. Salicylic acid (SA), chemically identified as 2-hydroxybenzoic acid, is regarded as a highly effective biotic elicitor that regulates a wide range of physiological and developmental processes in plants. Its influence extends to seed germination, stomatal regulation, pigment formation, photosynthetic efficiency, ethylene production, enzymatic activity, nutrient assimilation, floral induction, and membrane stability. Moreover, SA has been widely recognized for its contribution to improving plant tolerance against various abiotic stresses, including salinity, drought, and elevated temperatures. Exogenous application of salicylic acid has also been reported to enhance the accumulation of bioactive compounds in several plant species, such as sweet basil, peppermint, marjoram, amaranth, and Ammi visnaga [2].

## Materials and Method

The experimental data was conducted under the field in 2024–2025growing season at Research Station of College of Agriculture, University of Tikrit. Chemical analyses related to the research were performed at the laboratories of the Department of Field Crops, College of Agriculture Table 1. Characterization of experimented soil used before sowing.

**Table 1.** Physical and chemical properties of the experimental soil prior to planting at the Research Station of the College of Agriculture, University of Tikrit, 2024–2025.

| Analytical Category | Parameter | Unit / Symbol | Estimated Value | Method of Determination |
|---|---|---|---|---|
| Physical Properties | Sand | % | 16.40 | Hydrometer Method |
| | Silt | % | 54.10 | Hydrometer Method |
| | Clay | % | 29.50 | Hydrometer Method |
| | Soil Texture | - | Silt Loam | USDA Triangle |
| | Bulk Density | Mg $m^{-3}$ | 1.38 | Core Method |
| Chemical Properties | Soil Reaction (pH) | 1:2.5 (Susp.) | 7.75 | pH-meter |
| | Electrical Conductivity (ECe) | dS $m^{-1}$ | 3.45 | Electrical Conductivity |

| | | | | |
|---|---|---|---|---|
| Mineral Components | Organic Matter (O.M.) | g $\text{kg}^{-1}$ | 8.20 | Walkley-Black |
| | Available Nitrogen (N) | mg $\text{kg}^{-1}$ | 22.40 | Kjeldahl |
| | Available Phosphorus (P) | mg $\text{kg}^{-1}$ | 9.15 | Olsen Method |
| | Soluble Potassium (K) | mg $\text{kg}^{-1}$ | 165.0 | Flame Photometer |
| | Gypsum Content ($CaSO4$) | % | 14.20 | Acetone Method |
| | Total Carbonates ($CaCO3$) | % | 26.30 | Titration |
| | Cation Exchange Capacity (CEC) | $\text{cmol}_c\ \text{kg}^{-1}$ | 18.60 | Ammonium Acetate |

Seeds of a local sweet basil (Ocimum basilicum L.) cultivar were sown in 5-liter plastic pots filled with a soil and perlite mixture (2:1 v/v). Treatments commenced during the second week following seedling stabilization.

**2. Experimental Design and Treatments**

The experiment followed a Randomized Complete Block Design (RCBD) arranged in a two-factor factorial scheme with three replications. The treatments involved two main factors:

- **Factor A (Salinity Stress Levels):** 0 dS $m^{-1}$ (S0, Control), 4 dS $m^{-1}$ (S1), 8 dS $m^{-1}$ (S2), and 12 dS $m^{-1}$ (S3).
- **Factor B (Salicylic Acid Concentrations):** 0 mM (SA0, Control), 1 mM (SA1), and 2 mM (SA2).

Salicylic acid was applied as a foliar spray at a volume of 50 mL $\text{plant}^{-1}$ twice: first at two weeks prior to the induction of salinity stress, and second at three weeks post-induction.

**3. Measurements and Data Collection**

***Vegetative Growth Parameters***

A graduated ruler was used to measure the plant's height (in centimeters) from the base of the stem to the tip of the shoot during different phases of growth. The number of major branches on each plant was manually counted at harvest. The leaf disc method was used to gravimetrically determine leaf area. Leaf discs were taken with a cork borer of known surface area, weighed and compared to total leaf weight by the formula:

$$\text{Leaf Area} = (\text{Total Leaf Weight} / \text{Weight of Discs}) \times \text{Area of Discs}$$

Fresh weight was recorded immediately upon harvest, and dry weight was obtained after oven-drying the samples at 70°C until a constant mass was reached.

***Physiological and Biochemical Indicators***

Total chlorophyll content 1 g of fresh leaves was homogenised in 20 mL of 80% acetone, the extract was filtered and the final volume was adjusted to 50 mL (Lichtenthaler & Wellburn, 1983). The absorbance was measured at 663 nm and 645 nm in a spectrophotometer. Quantitative content was determined by:

$$\text{Chlorophyll } a\ (\text{mg g}^{-1}) = [(12.7 \times D663) - (2.69 \times D645)] \times V / (1000 \times W)$$

$$\text{Chlorophyll } b\ (\text{mg g}^{-1}) = [(22.9 \times D645) - (4.68 \times D663)] \times V / (1000 \times W)$$

$$\text{Total Chlorophyll} = \text{Chlorophyll } a + \text{Chlorophyll } b$$

(Where D = Optical density/absorbance, V = Extract volume in mL, and W = Leaf tissue fresh weight in grams).

Total Soluble Protein: 0.5 g leaf tissue was incubated in 10 mL 0.5 N NaOH at 4°C for 48 h. The volume was made up to 50 mL after filtration. Spectrophotometric quantification was performed with Bradford or Biuret assays using a standard curve of Bovine Serum Albumin (BSA). Activities of antioxidant enzymes (SOD and CAT) The enzyme extraction was carried out by grinding 0.5 g of leaf tissue in liquid nitrogen and homogenised in potassium phosphate buffer (pH 7.8) containing EDTA and PVP. The supernatant was separated by centrifugation as per the standard protocol (Bio-Protocol, 2017). Superoxide Dismutase (SOD) activity was determined by the inhibition of nitroblue tetrazolium (NBT) photochemical reduction at 560 nm in the presence of riboflavin. Catalase (CAT) activity was determined at 240 nm by measuring the rate of H2O2 decomposition with time. Enzyme activities were expressed as U mg-1 protein or µmol H2O2 min-1 g-1 FW.

### *HPLC-DAD Analysis of Phenolic Compounds and Flavonoids*

Phenolic metabolites were extracted with 80% methanol. The chromatographic separation was performed using a C18 analytical column (250 × 4.6 mm). The mobile phase was a gradient of water + 0.1% formic acid/acetonitrile and flow rate was constant 1 mL min −1 . Phenolic acids were detected at 280 nm and flavonoids at 370 nm.

## 4. Statistical Analysis

Data were statistically analyzed using SAS software through a two-way analysis of variance (ANOVA). Treatment means were subsequently compared using Duncan’s Multiple Range Test at a significance level of $P \leq 0.05$.

## Results

Increasing levels of salinity stress caused a progressive and statistically significant decline across all measured vegetative growth attributes (Table 2). Fresh leaf mass decreased from 62.5 g plant$^{-1}$ in the control to 19.4 g plant$^{-1}$ under the highest salinity level, representing a 68.9% reduction. Foliar application of SA (1 mM) significantly alleviated this osmotic inhibition, raising fresh weight from 32.1 to 41.5 g plant$^{-1}$ at 8 dS m$^{-1}$ (a 29.3% improvement).

**Table 2.** Sweet basil (*Ocimum basilicum L.*) vegetative development metrics (plant height, fresh weight, dried weight, and number of branches) are affected by salt stress levels and salicylic acid concentrations. According to Duncan's Multiple Range Test, means that are followed by the same letter in each column do not differ substantially at $P \leq 0.05$.

| Treatment | Plant Height (cm) | Fresh Weight (g/plant) | Dry Weight (g/plant) | Number of Branches |
|---|---|---|---|---|
| S0 + SA0 (Control) | 38.2 a | 62.5 a | 8.10 a | 7.2 a |
| S0 + SA1 | 40.1 a | 65.8 a | 8.54 a | 7.8 a |
| S1 + SA0 | 31.4 b | 47.3 b | 6.21 b | 5.6 b |
| S1 + SA1 | 35.6 ab | 54.9 ab | 7.14 ab | 6.5 ab |
| S2 + SA0 | 24.7 c | 32.1 c | 4.18 c | 4.1 c |
| S2 + SA1 | 29.8 bc | 41.5 bc | 5.40 bc | 5.2 bc |
| S3 + SA0 | 18.2 d | 19.4 d | 2.52 d | 2.8 d |
| S3 + SA1 | 23.5 cd | 27.6 cd | 3.59 cd | 3.7 cd |
| LSD 0.05 | 5.2 | 8.3 | 0.97 | 0.8 |

The total chlorophyll content under stress conditions decreased from 2.08 to 1.01 mg g$^{-1}$ (Table (3)).

**Table 3.** Effect of salinity stress levels and salicylic acid concentrations on total chlorophyll content (mg $g^{-1}$ FW) and proline accumulation (μmol $g^{-1}$ FW) in sweet basil leaves. Within each column, means assigned the same letter are not significantly different at $P \le 0.05$ based on Duncan's Multiple Range Test.

| Treatment | Total Chlorophyll (mg/g) | Proline (μmol/g) |
|---|---|---|
| S0 + SA0 | 2.08 a | 4.21 d |
| S0 + SA1 | 2.14 a | 4.45 d |
| S1 + SA0 | 1.78 b | 9.34 c |
| S1 + SA1 | 1.91 ab | 8.12 c |
| S2 + SA0 | 1.42 c | 17.56 b |
| S2 + SA1 | 1.63 bc | 14.23 b |
| S3 + SA0 | 1.01 d | 28.43 a |
| S3 + SA1 | 1.22 cd | 23.18 a |
| LSD 0.05 | 0.23 | 2.41 |

### 3. HPLC Quantitation of Phenolic Compounds

The HPLC-DAD analysis showed a significant increase in individual and total phenolic compounds with increasing salinity which was stress dependent (Table 4). The interaction treatment (S2 + SA1) had the highest accumulated concentration of all the phenolic fractions evaluated, giving a total phenolic value of 4924.7 mg kg-1 dry weight, even higher than that of the severe salinity treatment (S3) without SA.

**Table 4.** HPLC-DAD quantitative analysis of individual and total phenolic compounds (mg $kg^{-1}$ DW) in sweet basil leaves as influenced by salinity stress and salicylic acid foliar application. (*) denotes the treatment with the highest total phenolic accumulation. At $P \le 0.05$, means that are followed by the same letter in each column do not differ substantially.

| Treatment | Rosmarinic | Cichoric | Chlorogenic | Caffeic | Total Phenolics |
|---|---|---|---|---|---|
| S0 + SA0 (Control) | 788.6 e | 2252.7 d | 17.7 d | 67.1 d | 3212.4 e |
| S0 + SA1 | 863.4 de | 2410.3 cd | 19.4 d | 74.8 cd | 3418.7 de |
| S1 + SA0 | 895.3 cd | 2489.4 cd | 21.6 cd | 78.4 cd | 3557.6 cd |
| S1 + SA1 | 1024.7 bc | 2748.6 bc | 25.8 bc | 91.2 bc | 4012.3 bc |
| S2 + SA0 | 1068.4 bc | 2895.3 b | 28.4 b | 98.7 bc | 4268.1 bc |
| S2 + SA1 * | 1246.8 a | 3214.6 a | 34.7 a | 118.4 a | 4924.7 a |
| S2 + SA2 | 1148.3 ab | 3042.8 ab | 31.2 ab | 107.6 ab | 4598.3 ab |
| S3 + SA0 | 1124.6 ab | 2978.4 ab | 29.8 ab | 103.2 ab | 4428.7 ab |
| S3 + SA1 | 1198.4 a | 3124.7 a | 33.1 a | 112.8 a | 4742.3 a |
| LSD 0.05 | 142.4 | 318.6 | 4.8 | 19.7 | 412.3 |

### 4. HPLC Quantitation of Flavonoids

Total flavonoid content was significantly increased from 24.8 mg kg-1 dry weight in control to a maximum of 52.3 mg kg-1 dry weight at the interaction of (S2 + SA1) which was an increase of 110.9% (Table 5).

T**able 5.** HPLC-DAD quantitative analysis of individual flavonoids (quercetin and rutin) and total flavonoid content (mg $kg^{-1}$ DW) in sweet basil leaves as affected by salinity stress and salicylic acid application. (*) denotes the treatment with the highest total flavonoid accumulation. At $P \leq 0.05$, means that are followed by the same letter in each column do not differ substantially.

| Treatment | Quercetin | Rutin | Total Flavonoids |
|---|---|---|---|
| S0 + SA0 (Control) | 12.3 c | 8.4 d | 24.8 d |
| S0 + SA1 | 13.8 c | 9.1 cd | 27.4 cd |
| S1 + SA0 | 15.6 c | 9.8 cd | 29.7 cd |
| S1 + SA1 | 19.2 bc | 11.4 bc | 35.8 bc |
| S2 + SA0 | 21.4 bc | 11.9 bc | 38.2 bc |
| S2 + SA1 * | 31.6 a | 14.8 a | 52.3 a |
| S2 + SA2 | 26.8 ab | 13.2 ab | 45.1 ab |
| S3 + SA0 | 24.7 ab | 12.8 ab | 42.4 ab |
| S3 + SA1 | 28.9 a | 13.9 a | 48.7 a |
| LSD 0.05 | 7.4 | 1.6 | 9.8 |

## Discussion

The results confirmed the finding of Ciriello et al. It was also lower than that reported in Salma et al. [6] where had a decrease of fresh biomass 51.54% under salt stress. This dehydration of the root lowers water uptake as well as producing poisonous compounds of Cl- and Na+ ions in plant tissues that reduce metabolic pathways [7]. A study by Salehi et al. [8] reported the severe growth retardation of Ocimum basilicum when salinity is more than 4 dS m-1 and importance of organic regulators like salicylic acid for enhanced of vegetative vigour. Salinity is responsible for stomatal closure and decline in chlorophyll retention thereby affecting dry matter accumulation. The exogenous salicylic acid helps the plant to accumulate compatible osmolytes such as soluble sugars and proline to maintain the cellular turgor pressure. Moreover, salicylic acid serve as an antioxidant agent, preventing lipid peroxidation and loring oxidative stress accumulation [9].

Under stress conditions total chlorophyll content decreased from 2.08 to 1.01 mg g-1. This may be due to activation of the enzyme chlorophyllase and inhibition of enzymes involved in chlorophyll biosynthesis induced by ion toxicity [10]. Meanwhile, the accumulation of proline increased from 4.21 to 28.43 μmol g-1 . Proline is an important osmoprotectant to protect protein structures and cell membrane structures from dehydration [7], [11].

The exogenous SA significantly modulated the parameters that indicated the role of salicylic acid in alleviating oxidative stress. Total chlorophyll increased from 1.42 to 1.63 mg g-1 at S2 level by application of salicylic acid with stabilisation of proline requirements. This suggests that SA improves the efficiency of stress management without the plant having to invest a large amount of metabolic energy in the production of hyper-osmolyte. Salinity-induced distribution of photosynthetic process results in the rerouting of carbon precursors to the shikimate pathway for biosynthesis of antioxidant phenolics [12]. salicylic acid function as an elicitor signalling molecule and upregulates the expression of the PAL enzyme resulting in substainal accumulation of phenolics that can be detected by HPLC analysis [13].

The synergistic accumulation induced by salicylic acid results from the up-regulation of phenylpropanoid pathway and improved PAL activity [14], [15]. Salicylic acid enables the plant to maximise its defensive capabilities under stressful conditions and helps to stabilise root nutrient uptake, which in turn provides the essential carbon skeletons for the biosynthesis of secondary metabolites. [12]

Chlorophyll limitations and proline accumulation shift carbohydrate allocations to the phenylpropanoid pathway. This complies with the hypotheses of Ciriello et al. [16] that salinity acts as an elicitor to enhance the quality of secondary metabolites. Another study by Al-Huqail et al. [14] reported that salicylic acid balances the defensive metabolic costs and reduces osmotic lesions, resulting in the peak product performance during environmental challenges. Nasiri et al. [15] found a synergistic relationship between foliar biostimulants and salt applications in enhancing biochemical parameters.

## Conclusion

Salinity stress significantly suppresses vegetative biomass development and growth indices in sweet basil. Exogenous foliar application of salicylic acid at 1 mM functions as an effective biochemical elicitor, mitigating biomass loss and driving higher concentrations of targeted antioxidant metabolites.

## REFERENCES

[1] S. A. Almatroodi, M. A. Alsahli, A. Almatroudi, and A. H. Rahmani, “Ocimum sanctum: role in diseases management through modulating various biological activity,” *Pharmacognosy Journal*, vol. 12, no. 5, pp. 1198–1205, 2020.

[2] P. Rithichai, Y. Jirakiattikul, R. Nambuddee, and A. Itharat, “Effect of salicylic acid foliar application on bioactive compounds and antioxidant activity in holy basil (Ocimum sanctum L.),” *International Journal of Agronomy*, vol. 2024, no. 1, p. 8159886, 2024.

[3] M. Zaman, S. A. Shahid, L. Heng, S. A. Shahid, M. Zaman, and L. Heng, “Soil salinity: historical perspectives and a world overview of the problem,” in *Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques*, 2018, pp. 43–53.

[4] A. Yilmaz, “Vermicompost enhances salinetolerance in peanut (Arachis hypogaea L.),” *BSJ Agric*, vol. 6, no. 1, pp. 1–7, 2023.

[5] E. Van Zelm, Y. Zhang, and C. Testerink, “Salt tolerance mechanisms of plants,” *Annu. Rev. Plant Biol.*, vol. 71, pp. 403–433, 2020.

[6] H. Amer, N. Salman, M. Hawes, M. Chaqfeh, L. Mihaylova, and M. Mayfield, “An improved simulated annealing technique for enhanced mobility in smart cities,” *Sensors*, vol. 16, no. 7, p. 1013, 2016, doi: 10.3390/s16071013.

[7] Y. Arif, P. Singh, H. Siddiqui, and others, “Salicylic acid mediated ceasing of oxidative stress in plants under environmental trials,” *Front. Plant Sci.*, vol. 11, p. 40, 2020, doi: 10.3389/fpls.2020.00040.

[8] M. Salehi and others, “Exogenous salicylic acid improves growth and essential oil yield of sweet basil under salt stress by modulating antioxidant activity,” *Ind. Crops Prod.*, vol. 160, 2021.

[9] B. Ghahremani, S. Hassannejad, K. Alizadeh, and B. P. Eslam, “Salicylic acid alleviates oxidative stress and lipid peroxidation caused by clopyralid herbicide in Indian mustard plants,” *Acta Physiol. Plant.*, vol. 44, no. 4, 2022, doi: 10.1007/s11738-022-03382-x.

[10] S. Ahmadi and others, “Physiological and biochemical responses of basil to salt stress and salicylic acid treatments,” *J. Plant Growth Regul.*, 2023.

[11] A. M. Sofie, R. H. Khalil, and A. S. Al-Bakri, “Synergistic effects of salicylic acid on osmoregulation and proline metabolism in Ocimum basilicum under salinity stress,” *International Journal of Plant Science and Ecology*, vol. 12, no. 2, pp. 45–58, 2024.

[12] H. H. H. Ali, “The importance of the four English language skills: Reading, writing, speaking, and listening in teaching Iraqi learners,” *Humanitarian and Natural Sciences Journal*, vol. 3, no. 2, pp. 154–165, 2022, doi: https://doi.org/10.53796/hnsj3210.

[13] X. Wang, “Algorithmic discrimination: Examining its types and regulatory measures with emphasis on US legal practices,” *AI and Ethics*, vol. 4, pp. 1053–1067, 2024, doi: 10.1007/s43681-023-00332-y.

[14] A. A. Al-Huqail, R. M. El-Dakak, M. N. Sanad, and others, “Effect of salicylic acid foliar application on bioactive compounds and antioxidant activity in Holy Basil (Ocimum sanctum L.),” *International Journal of Agronomy*, p. 8159886, 2024, doi: 10.1155/2024/8159886.

[15] Y. Nasiri, H. A. Asadi-Gharneh, and M. Nasr-Isfahani, “Effects of NaCl and salicylic acid on basil phenolics and flavonoids,” *Journal of Plant Process and Function*, vol. 9, no. 37, pp. 1–14, 2020.

[16] M. Ciriello, L. Formisano, M. C. Kyriacou, and others, “Morpho-physiological and biochemical responses of hydroponically grown basil cultivars to salt stress,” *Antioxidants*, vol. 11, p. 2207, 2022, doi: 10.3390/antiox11112207.