



Synergistic Influence of Foliar Bio-stimulants and Salicylic Acid on the Vegetative Growth and Phytochemical Profile of *Citrus limon* L. Saplings

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Abstract

This study was conducted to evaluate the response of Lemon (*Citrus limon* L.) saplings to the exogenous application of seaweed extract and Salicylic Acid (SA), both independently and in combination, focusing on vegetative growth parameters and foliar chemical composition. The experiment was carried out at the nursery of the College of Agriculture, University of Samarra, during the 2024–2025 growing season (October to May). A Randomized Complete Block Design (RCBD) was employed in a factorial arrangement with two factors at three levels each. The results demonstrated that the synergistic treatment (Seaweed Extract + Salicylic Acid) significantly outperformed other treatments, yielding the highest values for vegetative development and nutrient accumulation. Specifically, plant height, stem diameter, leaf count per sapling, and leaf area reached 40.72 cm, 3.30 mm, 65.41 leaves, and 28.80 cm², respectively. Furthermore, the findings indicated a marked increase in the foliar concentration of macronutrients. These results highlight the potential of using integrated bio-stimulants to enhance the growth vigor and the endogenous levels of Nitrogen (N), Phosphorus (P), Potassium (K), and carbohydrates in lemon nursery stock. This study underscores the critical role of natural extracts and phytohormones in optimizing the performance of citrus saplings under nursery conditions.

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1. Introduction

Citrus species, belonging to the Rutaceae family, represent one of the most economically significant evergreen fruit groups globally. Among these, the lemon (*Citrus limon* Burmann) stands out due to its high concentration of ascorbic acid (Vitamin C), antioxidants, and essential oils, which are vital for the pharmaceutical, nutraceutical, and cosmetic industries ^[1]. However, the cultivation of high-quality citrus nursery stock is increasingly hindered by fluctuating climatic conditions and various abiotic stressors ^[2]. As modern agricultural markets demand sustainable and chemical-free production, there is an urgent need to develop eco-friendly strategies that enhance sapling vigor without compromising environmental integrity. Conventional fertilization, while effective for rapid nutrient delivery, often leads to long-term soil degradation, nutrient leaching—specifically nitrate (NO_3^-) contamination of groundwater—and the emission of potent greenhouse gases like nitrous oxide (N_2O) ^[3, 4, 6]. Such environmental implications, including the risks of eutrophication and Blue Baby Syndrome, have catalyzed the search for bioactive alternatives ^[5]. In this context, marine macroalgae extracts (e.g., *Ascophyllum nodosum*) and Salicylic Acid (SA) have emerged as promising candidates. Seaweed extracts are rich in natural phytohormones (auxins, cytokinins), amino acids (such as Glycine Betaine), and essential micronutrients, which collectively act as metabolic triggers to improve photosynthetic efficiency and water-use capacity ^[13, 15]. Concurrently, Salicylic Acid—a phenolic signaling molecule—plays a pivotal role in modulating plant physiological responses, including stomatal movement, nutrient uptake, and the activation of Systemic Acquired Resistance (SAR) ^[7, 8]. By stimulating antioxidant enzymes and enhancing Rubisco activity, SA provides a robust defense mechanism against oxidative damage ^[9]. Foliar application has proven to be an exceptionally efficient delivery

method, bypassing soil-binding constraints and ensuring the immediate availability of nutrients during critical early growth stages [12, 14]. While the individual benefits of these biostimulants are documented in vegetable crops like tomatoes and peppers [16], empirical data regarding their synergistic interaction on citrus saplings remains scarce. Therefore, this study aims to evaluate the interactive effects of foliar-applied seaweed extract and salicylic acid on the morphological development and chemical profile of Citrus limon L. saplings. The findings are expected to provide a sustainable framework for optimizing citrus nursery management and reducing reliance on synthetic mineral fertilizers.

Materials and Methods

Experimental Site and Plant Material

The experiment was conducted under the controlled environment of a lath house at the College of Agriculture, University of Samarra, during the 2024–2025 growing season (extending from October 2024 to May 2025). A total of 27 two-year-old lemon (*Citrus limon* L.) saplings were meticulously selected based on their morphological uniformity to ensure experimental consistency.

Initially grown in 5 kg plastic containers, the saplings were transplanted into larger 15 kg plastic pots filled with a standardized loamy soil medium.

To maintain optimal growth conditions, all conventional horticultural practices, including scheduled irrigation and integrated pest management (IPM), were strictly implemented. Weed control and phytosanitary treatments against insects and pathogens were applied following the recommended regional protocols and dosages.

Experimental Design and Treatments

The experiment was structured according to a Randomized Complete Block Design (RCBD) in a factorial arrangement (3×3). The study involved two primary factors:

1. Seaweed Extract (Factor A): Applied at three concentration levels: 0 (control), 50, and 100 ml L⁻¹.
2. Salicylic Acid (Factor S): Applied at three concentration levels: 0 (control), 50, and 100 mg L⁻¹.

The combination of these factors resulted in 9 unique treatment interactions. Each treatment was replicated three times, with one sapling per experimental unit, totaling 27 saplings (Al-Muhammadi & Al-Muhammadi, 2012).

The foliar applications were administered in three sequential stages. The initial application commenced on March 12, 2025, followed by the second and third applications at 21-day intervals. The specific treatment codes and concentrations are detailed in the table below:

Table 1:

R ₁	R ₂	R ₃
A ₀ S ₀	A ₂ S ₀	A ₂ S ₁
A ₀ S ₁	A ₁ S ₂	A ₀ S ₀
A ₀ S ₂	A ₂ S ₁	A ₁ S ₁
A ₁ S ₀	A ₀ S ₁	A ₁ S ₂
A ₁ S ₁	A ₂ S ₂	A ₀ S ₁
A ₁ S ₂	A ₀ S ₀	A ₀ S ₂
A ₂ S ₀	A ₁ S ₀	A ₂ S ₂
A ₂ S ₁	A ₀ S ₂	A ₂ S ₀

Measurements and Data Collection

Vegetative Growth Parameters

Morphological measurements were recorded at the commencement (March 2025) and conclusion (May 2025) of the experimental period. The net increase for each parameter was calculated as follows:

- **Plant Height (cm):** Measured from above the graft union using a graduated tape. The growth increment was determined by subtracting the initial height from the final height.
- **Stem Diameter (mm):** Measured 5 cm above the graft union using a digital vernier caliper.
- **Leaf and Branch Counts:** The total number of newly developed leaves and primary branches per sapling was recorded to determine the net increase during the growing season.

Chemical Composition Analysis

Foliar samples were collected from each experimental unit and oven-dried at 65°C for 72 hours until a constant weight was achieved. The dried tissues were finely ground, and 0.2

g of the resulting powder was digested using a concentrated acid mixture (\$H_2SO_4\$ and \$HClO_4\$) following the protocol of Jones and Steyn (1973).

- **Total Carbohydrates (%):** Quantified according to the method described by Joslyn (1970).
- **Nitrogen (N) Content (%):** Determined via the micro-Kjeldahl method (Jones & Steyn, 1973).
- **Potassium (K) Content (%):** Measured using a flame photometer (Elico CL-378) as per Bhargava and Raghupathi (1999).
- **Phosphorus (P) Content (%):** Determined colorimetrically via spectrophotometry (EMC V-LAB 100) at a wavelength of 410 nm.

Results and Discussion

Impact of Treatments on Vegetative Growth

The data presented in Table 1 indicate that foliar application of marine seaweed extract significantly enhanced all studied morphological parameters—including plant height, stem diameter, leaf count, and leaf area—compared to the control group. The bio-stimulatory effect was dose-dependent, with

the maximum concentration (100 ml L⁻¹) yielding the most superior results: plant height (40.72 cm), stem diameter (3.30 mm), leaf count (65.41 leaves), and leaf area (28.80 cm²). Similarly, exogenous treatment with Salicylic Acid (SA) at 100 mg L⁻¹ exerted a significant positive influence on growth traits, resulting in values of 36.22 cm, 3.24 mm, 61.33 leaves, and 26.20 cm² for the aforementioned parameters,

respectively.

Statistical analysis revealed a highly significant interaction ($P < 0.05$) between the highest concentrations of seaweed extract and salicylic acid. This synergistic effect underscores the efficacy of combining metabolic triggers and signaling molecules to optimize the physiological performance and vegetative vigor of Citrus limon L. nursery stoc

Table 2: Effect of seaweed oil and silicic acid on some vegetative growth characteristics of lemon seedlings (Citrus limon Burmann)

SWE (ml L ⁻¹)	SA (mg L ⁻¹)	Plant Height (cm)	Stem Diameter (mm)	Leaf Number	Leaf Area (cm ²)
0.00	0.00	20.70	1.45	35.70	13.40
50	0.00	24.12	2.50	42.11	16.15
50	50	33.91	2.71	51.71	20.30
100	0.00	30.63	2.90	55.40	20.33
100	100	36.22	3.24	61.33	26.20
150	0.00	25.90	3.16	60.01	18.87
150	150	40.72	3.30	65.41	28.80

Least significant difference (LSD) at a probability level of 0.05:

Trait	Seaweed Extract (SWE)	Salicylic Acid (SA)	Interaction (SWE × SA)
Plant Height (cm)	2.620	2.620	5.284
Stem Diameter (mm)	5.273	5.273	5.559
Leaf Number	2.866	2.866	5.925
Leaf Area (Scm ² \$)	2.253	2.253	4.542

Foliar Nutrient Composition (N, P, and K)

The chemical analysis of the leaves (Table 2) revealed a significant enhancement in the accumulation of primary macronutrients—Nitrogen (N), Phosphorus (P), and Potassium (K)—following the exogenous application of seaweed extract and Salicylic Acid. The data indicate a clear linear correlation between the treatment concentrations and the endogenous nutrient levels.

The synergistic interaction between the highest concentrations of the two factors (150 ml L⁻¹ SWE + 150 mg L⁻¹ SA) yielded the most superior chemical profile.

Specifically, Nitrogen levels reached 2.71%, Phosphorus 0.50%, and Potassium 1.76%. In stark contrast, the control group (A0S0) exhibited the lowest nutrient concentrations, recording values of 1.03% N, 0.09% P, and 0.60% K. Statistical significance was most pronounced in the combined treatments, suggesting that the integration of seaweed-derived biostimulants and phenolic signaling molecules (SA) facilitates a more efficient ion transport mechanism and nutrient assimilation within the citrus saplings.

Table 3: Effect of foliar seaweed extract and salicylic acid applications on the macronutrient content (N, P, K) of lemon (Citrus limon L.) leaves.

SWE (ml L ⁻¹)	SA (mg L ⁻¹)	N (%)	P (%)	K (%)
0.00	0.00	1.03	0.09	0.60
50	0.00	1.17	0.14	1.12
50	50	1.66	0.19	1.16
100	0.00	1.10	0.20	1.27
100	100	2.22	0.36	1.40
150	0.00	2.10	0.22	1.40
150	150	2.71	0.50	1.7

Least significant difference (LSD) at a probability level of 0.05:

elements	Seaweed Extract (SWE)	Salicylic Acid (SA)
N (%)	0.120	0.120
P (%)	0.400	0.400
K (%)	0.200	0.200

Discussion

The results obtained underscore the profound influence of seaweed extract (SWE) and Salicylic Acid (SA) on the vegetative architecture of Citrus limon L. saplings. The significant increases in plant height, stem diameter, leaf count, and leaf area suggest that these biostimulants provide a rich source of bioactive compounds that bolster

photosynthetic efficiency and stimulate primary tissue development. These substances act as metabolic catalysts, enhancing protein synthesis and modulating biochemical pathways essential for cell division and elongation. Our findings align with previous literature, attributing the enhancement of growth traits to the role of bio-organic materials in activating key enzymes responsible for the

endogenous biosynthesis of phytohormones, such as auxins, cytokinins, and gibberellins. These hormones indirectly influence chlorophyll formation and stabilize the photosynthetic apparatus. Furthermore, the observed increase in foliar carbohydrate content reflects a superior metabolic efficiency and improved photo-assimilate translocation within the plant system.

The elevated concentrations of Nitrogen (N), Phosphorus (P), and Potassium (K) in the treated leaves suggest that the synergistic application of SWE and SA enhances cell membrane permeability. This physiological adjustment facilitates more efficient nutrient uptake and assimilation, ultimately enriching the plant's nutritional status. While foliar application can sometimes face challenges regarding concentration gradients and distribution (18), the use of organic extracts and natural signaling molecules ensures rapid absorption and high bioavailability, as corroborated by contemporary citrus research (18).

Conclusions

This study demonstrates that the integrated foliar application of 150 ml L⁻¹ seaweed extract and 150 mg L⁻¹ Salicylic Acid significantly optimizes both the vegetative growth and chemical profile of Citrus limon L. saplings. The most robust responses were recorded during the peak growth seasons, leading to substantial improvements in plant height, stem girth, and foliar expansion. Moreover, this treatment effectively increased the leaf content of essential macronutrients (N, P, K) and carbohydrates. Consequently, this combined biostimulant protocol is recommended for nursery management to produce high-quality, vigorous citrus stock while moving towards more sustainable agricultural practices.

References

1. Bhardwaj RL, Pandey S. Juice blends—a way of utilization of under-utilized fruits, vegetables, and spices: a review. *Crit Rev Food Sci Nutr.* 2011;51(6):563–70.
2. Al-Khafaji MA. *Plant Growth Regulators and Their Horticultural Applications.* Baghdad: University Press for Printing, Publishing and Translation – Ministry of Higher Education – University of Baghdad – Iraq; 2014.
3. Tripathi DK, *et al.* Managing abiotic stress in citrus: recent advances and future perspectives. *Plants.* 2022;11(1):87.
4. Havlin JL, *et al.* *Soil Fertility and Fertilizers: An Introduction to Nutrient Management.* Pearson; 2013.
5. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. *Nature.* 2002;418(6898):671–7.
6. Camargo JA, Alonso Á. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environ Int.* 2006;32(6):831–49.
7. Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, *et al.* Seaweed extracts as biostimulants of plant growth and development. *J Plant Growth Regul.* 2009;28(4):386–99.

8. Khan MIR, Fatma M, Per TS, Anjum NA, Khan NA. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front Plant Sci.* 2015;6:462.
9. Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. *Environ Exp Bot.* 2010;68(1):14–25.
10. Craigie JS. Seaweed extract stimuli in plant science and agriculture. *J Appl Phycol.* 2011;23:371–93.
11. Sharma HSS, *et al.* Biostimulant activities of seaweed extracts in plants: implications for sustainable crop production. *Vegetos.* 2019;32:205–15.
12. Ahmed M, *et al.* Effect of seaweed extract on growth and fruit quality of citrus. *J Plant Nutr.* 2021;44(7):1020–32.
13. Fernández V, Sotiropoulos T, Brown P. *Foliar Fertilization: Scientific Principles and Field Practices.* Paris: International Fertilizer Industry Association (IFA); 2013.
14. Abdul Qadir N, Hassan Yousef N, Latif Al-Aithawi. *Soil Fertility and Fertilizers.* Baghdad: College of Agriculture, University of Baghdad, Ministry of Higher Education and Scientific Research; 1990.

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