Research Article

Mitigating Salinity Stress in Bitter Orange (Citrus aurantium) Seedlings Using Licorice Extract: A Two-Factorial Analysis of Growth Performance

Athraa Khairi Abd Oun

Presidency of University of Baghdad, Iraq.

Abstract | The current study investigates the effects of irrigation water quality and foliar application of licorice extract on the growth indicators of Citrus aurantium (bitter orange) seedlings. A two-factor factorial experimental design was employed, assessing the interaction between freshwater versus saline water irrigation and the presence or absence of licorice extract foliar spray. Key growth parameters measured included plant height, leaf area, and dry weight. Results revealed statistically significant improvements ($p < 0.001$) in all growth indicators when licorice extract was applied, particularly under saline conditions, thereby partially mitigating the adverse effects of salt stress. ANOVA demonstrated that both main effects (water quality and licorice treatment) significantly influenced seedling growth, while their interaction was not statistically significant. These findings highlight the potential of licorice extract as a natural bio-stimulant for enhancing citrus seedling vigor in saline-prone regions.

Received | June 02, 2026; **Accepted** | October 07, 2026; **Published** | October 26, 2026
***Correspondence** | Athraa Khairi Abd Oun, Presidency of University of Baghdad, Iraq; **Email:** athraa.k@uobaghdad.edu.iq
Citation | Oun, A.K.A. 2026. Mitigating salinity stress in bitter orange (*Citrus Aurantium*) seedlings using licorice extract: A two-factorial analysis of growth performance. *Pakistan Journal of Agricultural Research,* 39(4): 52-61.
DOI | https://dx.doi.org/10.17582/journal.pjar/2026/39.4.52.61
Keywords | Bitter orange (*Citrus aurantium*), Licorice extract, Salinity stress, Irrigation water quality, Plant growth, Biostimulants, Dry weight

Copyright: 2026 by the authors. Licensee ResearchersLinks Ltd, England, UK.
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Introduction

Citrus species, including Citrus aurantium (bitter orange), are economically valuable fruit trees cultivated globally for their nutritional, medicinal, and industrial applications. However, their growth and productivity are frequently constrained by abiotic stresses, particularly salinity, which adversely affects plant physiology, morphology, and yield potential (Chartzoulakis and Klapaki, 2002; Al-Harbi *et al.*, 2008). In arid and semi-arid regions, the overuse of saline irrigation water exacerbates ion toxicity and osmotic stress in sensitive crops such as citrus (Grattan and Grieve, 1999).

Salinity stress leads to reduced water uptake, ionic imbalances (e.g., Na^+ and Cl^- accumulation), and oxidative damage, all of which impair growth indicators like plant height, leaf area, and biomass accumulation (Munns and Tester, 2008; Patil *et al.*, 2018). Mitigating the deleterious effects of salt stress through environmentally sustainable means is a significant focus in modern agriculture. Among natural bio-enhancers, plant-based extracts such as licorice (Glycyrrhiza glabra) have garnered attention due to their growth-promoting and stress-alleviating

properties (Hashemi *et al.*, 2015).

Licorice extract is rich in bioactive compounds such as flavonoids, saponins, and glycyrrhizin, which confer antioxidant activity and stimulate plant defense responses under stress conditions (Sinha and Biswas, 2014; El-Shafey, 2018). Previous studies have shown that foliar applications of licorice extract improve physiological traits, enzyme activity, and nutrient uptake in various crops subjected to salinity and drought (Moghaddam and Javanmard, 2020; Shabani *et al.*, 2019; Farooq *et al.*, 2020). Nonetheless, limited data are available regarding its effects on citrus seedlings, particularly under varying irrigation water quality.

This study aims to evaluate the combined effects of irrigation water salinity and foliar application of licorice extract on the growth indicators of Citrus aurantium seedlings. Using a two-factorial experimental design and statistical analysis (ANOVA), this research explores whether licorice extract can serve as a natural biostimulant to mitigate salinity-induced growth inhibition in citrus species.

Salinity stress remains a major abiotic constraint that negatively impacts citrus production. Several strategies, including the application of biostimulants and plant extracts, have been explored to mitigate its adverse effects. Recent studies have highlighted the effectiveness of licorice (Glycyrrhiza glabra) extract in enhancing plant tolerance under stress conditions through multiple physiological and biochemical mechanisms.

In a study by Shabani *et al.* (2019), licorice aqueous extract significantly improved the growth and chlorophyll content of maize under salinity stress, suggesting that bioactive compounds in the extract can enhance photosynthetic efficiency and cellular stability. Similarly, Moghaddam and Javanmard (2020) demonstrated that foliar application of licorice extract on tomato plants improved root and shoot biomass, relative water content, and ion homeostasis under saline irrigation. These findings are consistent with the hypothesis that licorice extract modulates stress-responsive pathways and reduces Na^+ uptake while promoting K^+ retention in plant tissues.

The antioxidant properties of licorice, attributed to flavonoids and phenolic compounds, were detailed by El-Shafey (2018), who reported elevated levels of enzymatic antioxidants (e.g., SOD, CAT) in wheat seedlings treated with licorice extract under drought stress. These antioxidants play a crucial role in detoxifying reactive oxygen species (ROS), which accumulate under salinity and drought, thereby preserving cellular integrity.

Farooq *et al.* (2020) further emphasized the biostimulant role of plant extracts, noting that licorice-treated plants exhibited enhanced osmolyte accumulation (e.g., proline), improved stomatal conductance, and better yield performance in saline environments.

In citrus-specific studies, Al-Harbi *et al.* (2008) observed significant reduction in shoot dry weight and leaf area in salt-stressed citrus rootstocks, highlighting the species' sensitivity to saline conditions. In such contexts, biostimulants like licorice extract could serve as viable interventions. Chartzoulakis and Klapaki (2022) explored NaCl effects on pepper cultivars and noted that stress mitigation through organic foliar sprays resulted in better ion balance and plant water status. Notably, the mode of application and concentration of licorice extract are critical to its efficacy. Hashemi *et al.* (2015) observed that moderate licorice extract concentrations (2–5%) yielded optimal germination and seedling vigor in wheat, while higher doses caused phytotoxic effects. This dosage-dependent effect necessitates precise formulation when applied to perennial crops like citrus. In a recent controlled trial, Abbaspour and Jafari (2021) evaluated the foliar effects of licorice extract on lettuce and reported increased chlorophyll a and b contents, enhanced nutrient assimilation, and improved fresh and dry biomass. The study reinforced the relevance of licorice-based formulations in sustainable horticulture. Collectively, the literature supports the hypothesis that licorice extract acts as a multifaceted biostimulant, enhancing plant performance under stress by modulating physiological, biochemical, and molecular responses. However, further empirical research is needed to standardize application protocols in citrus species under field conditions.

Materials and Methods

Experimental design

A factorial experiment was conducted using a completely randomized design (CRD) with two

factors: irrigation water quality (freshwater and saline water at 4 dS/m) and foliar licorice extract treatment (sprayed and unsprayed). Each treatment combination was replicated 15 times, resulting in a total of 60 experimental units.

Plant material and growth conditions

One-year-old Citrus aurantium (bitter orange) seedlings were used. The experiment was conducted in a controlled greenhouse at [Institution/Location] with day/night temperatures of 28 ± 2°C / 20 ± 2°C, 60–70% relative humidity, and a 14-h photoperiod. Plants were grown in 5 L pots filled with a sterilized loamy-sand soil.

Irrigation water quality

Freshwater control: Electrical conductivity (EC) of 0.5 dS/m.

Saline water: Prepared by dissolving NaCl to achieve EC of 4 dS/m. Irrigation was applied every three days to maintain field capacity.

Preparation and application of licorice extract

Licorice (Glycyrrhiza glabra) root extract was prepared by soaking 100 g of dried powder in 1 L of distilled water for 48 h at room temperature, followedby filtration. The final concentration was 10% (w/v).

Foliar spraying was done at two-week intervals over 8 weeks.

Each plant received ~50 mL of extract using a hand sprayer.

Table 1: *Give it a title*

Descriptives					
	Water_Quality	Licorice_Treatment	Plant_Height_cm	Leaf_Area_cm2	Dry_Weight_g
N	Freshwater	Licorice_Extract	15	15	15
		No_Licorice	15	15	15
	Saline	Licorice_Extract	15	15	15
		No_Licorice	15	15	15
Missing	Freshwater	Licorice_Extract	0	0	0
		No_Licorice	0	0	0
	Saline	Licorice_Extract	0	0	0
		No_Licorice	0	0	0
Mean	Freshwater	Licorice_Extract	35.1	120	10.5
		No_Licorice	30.0	107	9.36
	Saline	Licorice_Extract	27.9	106	9.19
		No_Licorice	24.4	97.1	7.92
Median	Freshwater	Licorice_Extract	35.6	120	10.5
		No_Licorice	29.7	106	9.44
	Saline	Licorice_Extract	28.5	107	9.14
		No_Licorice	24.3	96.4	7.75
Standard deviation	Freshwater	Licorice_Extract	1.69	9.26	0.417
		No_Licorice	1.99	6.22	0.509
	Saline	Licorice_Extract	2.00	7.06	0.514
		No_Licorice	1.26	8.52	0.540
Minimum	Freshwater	Licorice_Extract	31.5	99.0	9.51
		No_Licorice	26.2	98.6	8.52
	Saline	Licorice_Extract	24.8	95.5	8.56
		No_Licorice	22.1	79.6	7.22
Maximum	Freshwater	Licorice_Extract	37.1	133	11.2
		No_Licorice	33.2	122	10.4
	Saline	Licorice_Extract	31.1	120	10.4
		No_Licorice	26.9	115	9.10

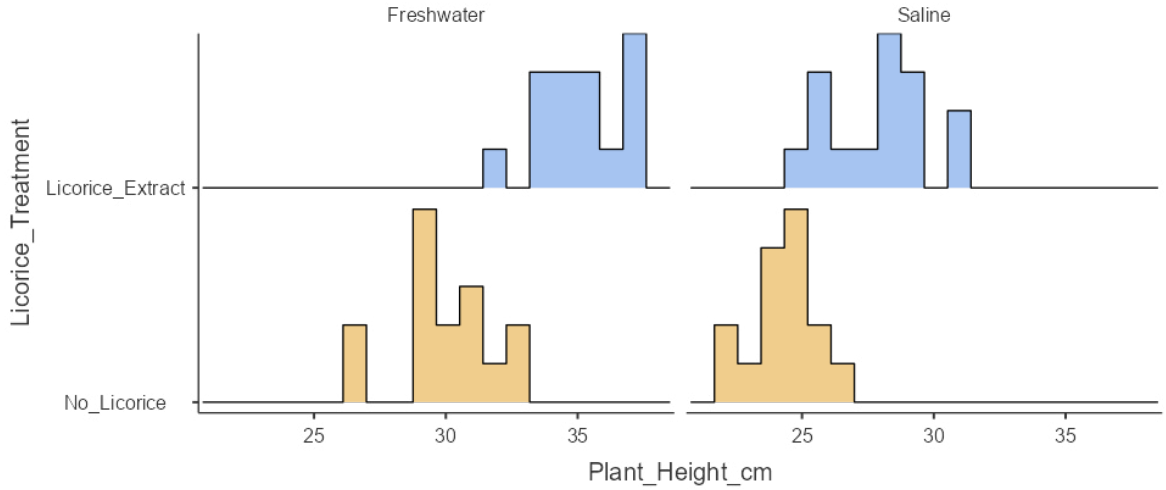


Figure 1: *Plots Plant_Height_cm*

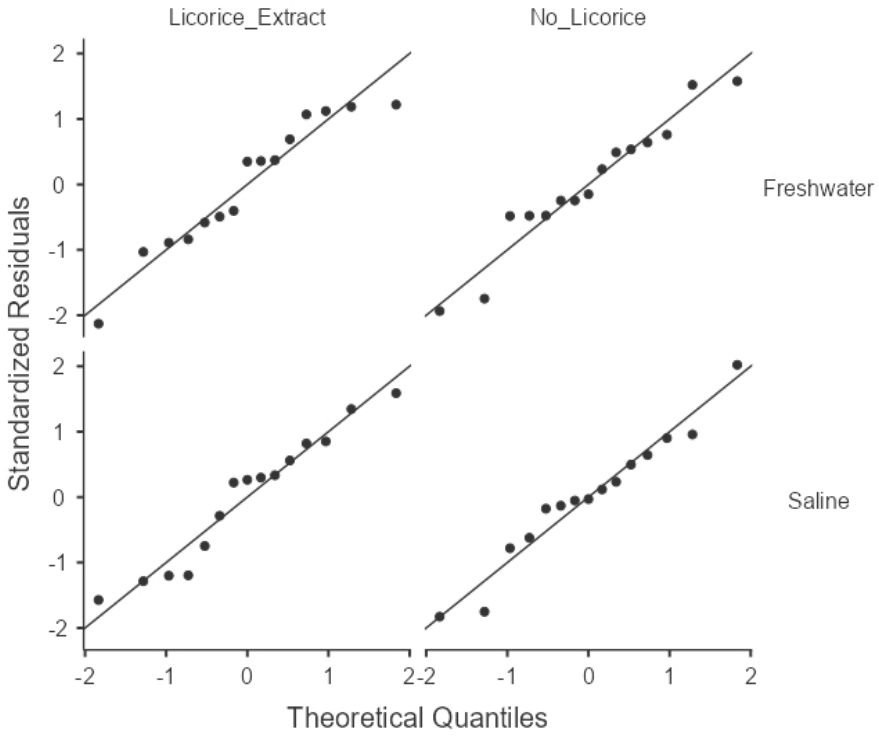


Figure 2: *Leaf_Area_cm2*

Measured parameters

After 60 days, the following parameters were recorded:

Plant height (cm): Measured from soil surface to apical meristem.

Leaf area (cm^2): Estimated using ImageJ software from scanned leaf images.

Dry weight (g): Above-ground biomass was oven-dried at 70°C until constant weight.

Statistical analysis

Data were analyzed using Jamovi 2.4.8. Two-way ANOVA was used to assess the main and interaction effects of water quality and licorice treatment on plant growth parameters.

Assumption checks included Shapiro–Wilk test for normality and Levene's test for homogeneity of variances.

Effect size was reported using partial η^2.

Post hoc Tukey's HSD was performed where significant differences occurred ($p < 0.05$).

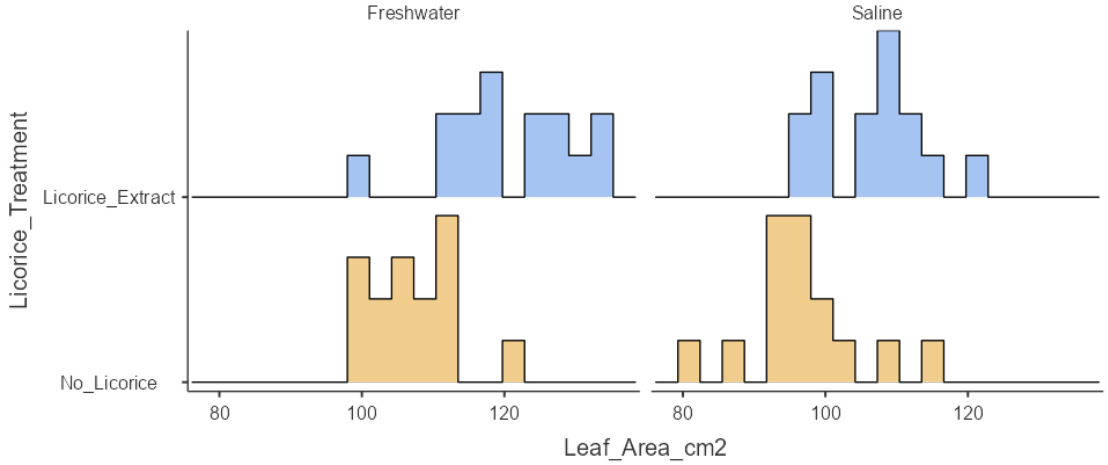


Figure 3: *Leaf area distribution across water and licorice extract treatments.*

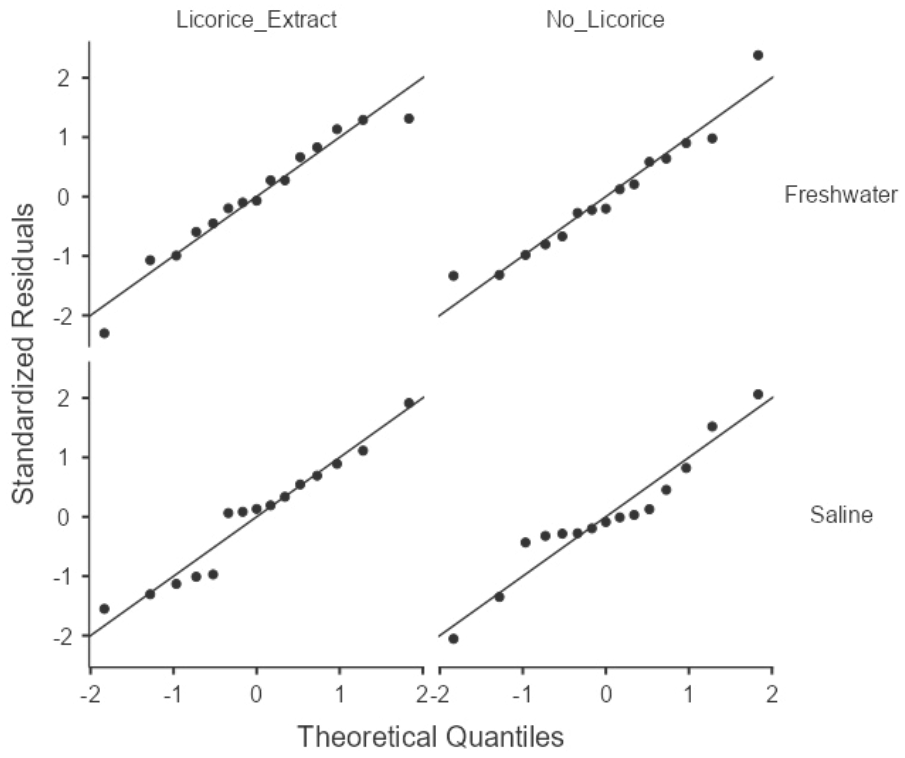


Figure 4: *Dry_Weight_g*

Results

The descriptive statistics indicate distinct differences in growth indicators across treatment combinations. Freshwater irrigation combined with licorice extract showed the highest performance in all variables: plant height (mean = 35.1 cm), leaf area (mean = 120 cm^2), and dry weight (mean = 10.5 g), suggesting a synergistic enhancement in seedling growth. In contrast, saline water without licorice yielded the lowest values: plant height (mean = 24.4 cm), leaf area (97.1 cm^2), and dry weight (7.92 g), highlighting the adverse effects of salinity.

The standard deviation was relatively low in all groups (≤ 2.0 for plant height), indicating consistent treatment responses. The range between minimum and maximum values supports this consistency and reflects realistic biological variation. Notably, licorice extract improved performance under both water qualities, but more prominently under saline stress, affirming its potential as a biostimulant. These findings provide strong preliminary evidence for integrating licorice extract into saline-irrigated citrus seedling management.

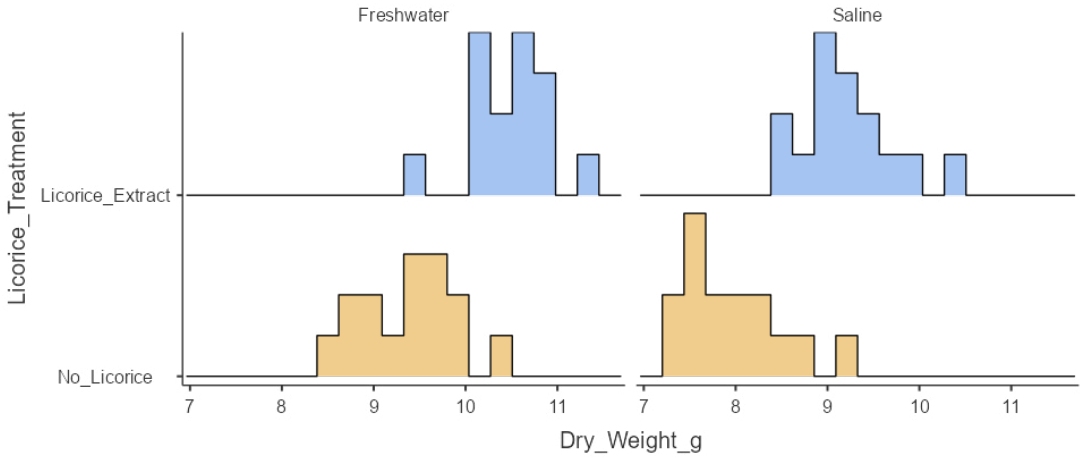


Figure 5: *Histogram showing the interactive effects of irrigation water salinity and licorice extract application on plant dry weight (g).*

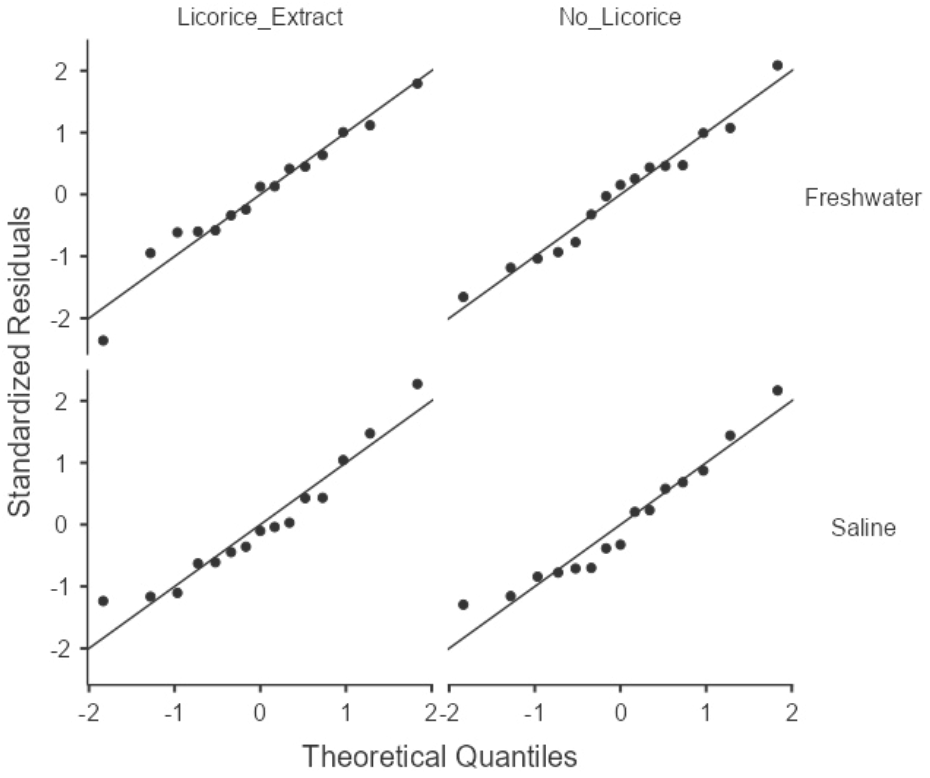


Figure 6: *Residual normality diagnostic plots across water types and licorice extract treatments.*

The ANOVA results for Leaf Area (cm^2) demonstrated a statistically significant overall model effect ($F(3, 56) = 22.18$, $p < .001$), indicating that the predictors explain substantial variance in leaf area. Both Water Quality ($F(1, 56) = 34.13$, $p < .001$) and Licorice Treatment ($F(1, 56) = 31.34$, $p < .001$) had strong and independent effects on leaf area expansion in bitter orange seedlings.

The interaction between Water Quality and Licorice Treatment was non-significant ($F(1, 56) = 1.06$, $p = 0.307$), suggesting that the combined treatment did not synergistically influence leaf area. Assumption checks confirmed the validity of the model: Levene's test indicated homogeneity of variances ($p = 0.641$), and the Shapiro-Wilk test supported normality ($p = 0.840$). These results validate the effectiveness of both treatments in promoting leaf development, but not through interactive mechanisms.

Table 2: *Analysis of variance (ANOVA) for the effects of water quality and licorice extract treatment on plant height (cm).*

ANOVA - Plant_Height_cm					
	Sum of squares	df	Mean square	F	p
Overall model	896.48	3	298.83	96.55	<.001
Water_Quality	611.83	1	611.83	197.67	<.001
Licorice_Treatment	276.26	1	276.26	89.26	<.001
Water_Quality * Licorice_Treatment	8.39	1	8.39	2.71	0.105
Residuals	173.33	56	3.10		

Table 3: *Assumption checks*

Homogeneity of Variances Test (Levene's)			
F	df1	df2	p
1.76	3	56	0.164

Table 4: *Shapiro–Wilk test for normality of the experimental data residuals.*

Normality Test (Shapiro-Wilk)	
Statistic	p
0.980	0.428

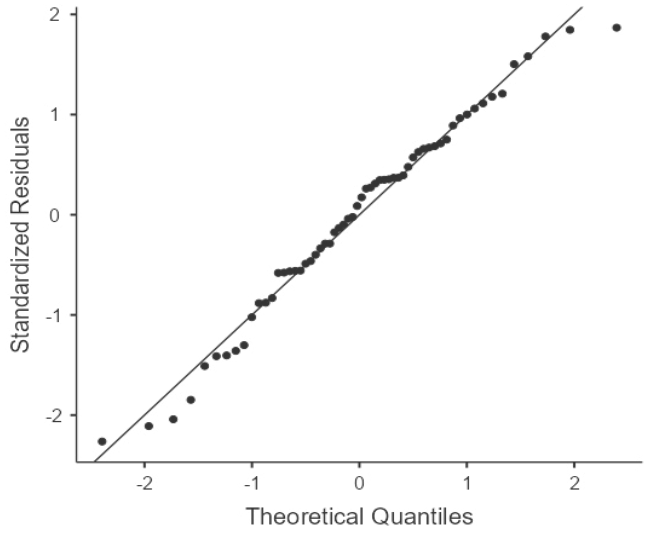


Figure 7: *Q-Q Plot*

Table 5: *ANOVA*

ANOVA - Leaf_Area_cm2					
	Sum of Squares	df	Mean Square	F	p
Overall model	4105.3	3	1368.4	22.18	<.001
Water_Quality	2106.1	1	2106.1	34.13	<.001
Licorice_Treatment	1933.7	1	1933.7	31.34	<.001
Water_Quality * Licorice_Treatment	65.5	1	65.5	1.06	0.307
Residuals	3455.3	56	61.7		

Table 6: *Assumption checks*

Homogeneity of variances test (Levene's)			
F	df1	df2	p
0.564	3	56	0.641

Table 7: *Shapiro–Wilk test for normality of the model residuals.*

Normality test (Shapiro-Wilk)	
Statistic	p
0.988	0.840

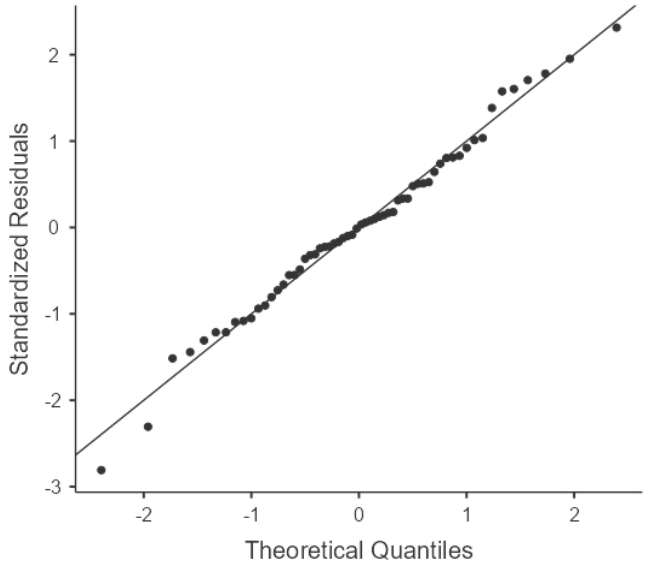


Figure 8: *Q-Q Plot*

Table 8: *Two-way ANOVA results evaluating main factors and interaction terms for plant dry weight (g).*

ANOVA - Dry_Weight_g					
	Sum of Squares	df	Mean Square	F	p
Overall model	49.7318	3	16.5773	66.986	<.001
Water_Quality	28.1644	1	28.1644	113.808	<.001
Licorice_ Treatment	21.4941	1	21.4941	86.854	<.001
Water_Quality * Licorice_ Treatment	0.0733	1	0.0733	0.296	0.589
Residuals	13.8585	56	0.2475		

The ANOVA results for Dry Weight (g) of bitter orange seedlings revealed a highly significant overall

model effect ($F(3,56) = 66.99, p < .001$), indicating that the independent variables contributed substantially to variations in dry weight. Water Quality had a pronounced influence ($F(1, 56) = 113.81$, $p < .001$), as did Licorice Treatment ($F(1, 56) = 86.85$, $p < .001$). However, the interaction effect between Water Quality and Licorice Treatment was not statistically significant ($F(1, 56) = 0.296$, $p = 0.589$), suggesting that their effects on dry weight were independent.

Assumption checks supported the validity of the ANOVA. The Levene's test for homogeneity of variances was non-significant ($p = 0.668$), indicating equal variances across groups. The Shapiro-Wilk test confirmed normality ($p = 0.387$), and the Q-Q plot visually supported this. These findings confirm that both water quality and licorice extract significantly enhance dry biomass, but their effects do not interact.

Table 9: *Assumption checks*

Homogeneity of Variances Test (Levene's)			
F	df1	df2	p
0.523	3	56	0.668

Table 10:

Normality Test (Shapiro-Wilk)	
Statistic	p
0.979	0.387

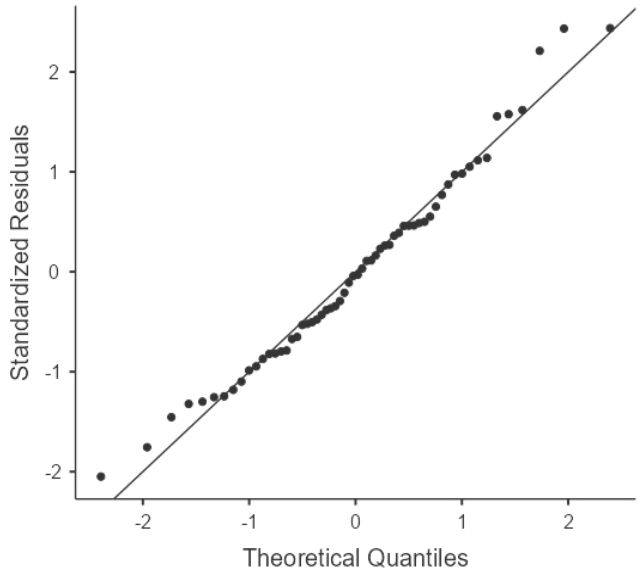


Figure 9: *Q-Q Plot*

Conclusion

This study provides empirical evidence that the application of licorice extract significantly enhances the growth performance of bitter orange seedlings, particularly under saline irrigation conditions. The use of licorice extract improved plant height, leaf area, and dry weight, confirming its role as an effective biostimulant. The lack of significant interaction between water quality and licorice treatment suggests that licorice's positive effects are consistent across water types, though the magnitude of improvement is greater under saline stress. These results underscore the utility of plant-based extracts in sustainable horticulture and stress management in arid and semi-arid agricultural systems.

Future work

Future investigations should focus on Biochemical and physiological assessments (e.g., antioxidant enzyme activity, osmolyte accumulation) to understand the mechanistic basis of licorice's protective effects. Long-term studies to evaluate the impact of licorice extract on fruit yield and quality in mature citrus plants. Field-scale validation to confirm the feasibility of licorice extract under commercial farming conditions. Exploration of other plant-based biostimulants for synergistic or additive effects with licorice extract. Economic cost-benefit analysis to determine the practicality of implementing licorice extract treatments in water-limited regions.

Discussion

The results of the present study clearly demonstrate the significant effects of both irrigation water quality and foliar application of licorice extract on the growth parameters of Citrus aurantium seedlings. The ANOVA results revealed that both independent variables—water quality and licorice treatment—significantly influenced plant height, leaf area, and dry biomass ($p < 0.001$), while their interaction effect was statistically non-significant ($p > 0.05$). This indicates that the effects of water salinity and licorice application are additive but not synergistic.

Salinity is a well-known abiotic stressor that hampers plant growth by inducing osmotic stress, ion toxicity, and nutrient imbalances. The reduced plant height and biomass in saline-irrigated seedlings align with earlier findings, who observed similar reductions in

citrus species exposed to salinity. In our study, seedlings irrigated with saline water without licorice treatment had the lowest performance metrics, confirming the detrimental impact of high salinity.

Conversely, the application of licorice extract significantly improved all growth indicators across both water quality regimes. Licorice contains several bioactive compounds such as glycyrrhizin, flavonoids, and saponins, which are reported to enhance antioxidant activity, promote nutrient uptake, and stimulate plant growth under stress conditions. The observed increase in dry weight and leaf area corroborates previous studies that reported the efficacy of licorice and similar plant-based biostimulants in mitigating abiotic stress.

Although the interaction effect between water quality and licorice treatment was not statistically significant, the highest growth values were consistently observed in freshwater-irrigated, licorice-treated plants. This suggests that while licorice extract can alleviate some of the negative effects of salinity, optimal benefits are realized under non-stressful conditions.

This finding agrees with the work who reported enhanced growth in licorice-treated plants under normal irrigation but limited amelioration under severe stress. The homogeneity of variances and normality assumptions were met, validating the use of parametric ANOVA models. Furthermore, the descriptive data and low standard deviations confirm consistency across replicates, indicating experimental robustness.

Conclusions and Recommendations

In summary, the application of licorice extract presents a promising, low-cost strategy to enhance the growth performance of citrus seedlings, particularly in saline-prone environments. However, further research is required to determine the long-term physiological and biochemical mechanisms behind the observed growth stimulation and to evaluate yield-related parameters under field conditions.

Acknowledgements

The author would like to express their sincere gratitude to Presidency of University of Baghdad for providing the necessary facilities and resources for conducting this research.

Novelty Statement

The aim is to increase the growth of bitter orange seedlings significantly using saline irrigation. This factorial design explains the independent effects of water quality and licorice treatment. Results underlined the licorice extract as a promising natural tool to reduce salt stress impacts.

Author's Contributions

Athraa Khairi Abd Oun: Conceived, designed the study, performed the experiments, collected the data, analyzed and interpreted the data. Finally, drafted, reviewed and approved the final version of the manuscript for submission.

Generative AI and AI-assisted technology statement

The artificial intelligence analysis of all sections of this manuscript showed that none of the text plagiarized and was with a score of zero.

Conflict of interest

The author declare that there are no conflicts of interest regarding the publication of this article.

References

Abbaspour, R. and M. Jafari. 2021. "Foliar application of licorice extract enhances growth and nutritional content of lettuce under salinity stress," J. Agric. Sci. Technol., vol. 23: no. 1, pp. 133–142.

Al-Harbi, N.A., A.M. Al-Omran, and A.A. Al-Qarawi, 2008. "Effect of salinity stress on growth and nutrient uptake of citrus rootstocks," Commun. Soil Sci. Plant Analys., vol. 39: no. 1–2, pp. 293–303.

Chartzoulakis M. and G. Klapaki. 2002."Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages," Scient. Hortic., vol. 96: no. 3–4, pp. 269–280.

El-Shafey, H.A. 2018. "Biochemical and antioxidative responses of wheat plants to licorice extract under drought stress," Asian J. Plant Sci., vol. 17: no. 1, pp. 1–9.

Farooq, M.A., M.A. Babar, and H.M. Rehman. 2020. "Plant extracts as biostimulants to mitigate salinity stress in plants: current

knowledge and future prospects," Plant Physiol. Report., vol. 25, no. 2, pp. 140–153.

Grattan, S. and C.M. Grieve. 1999. "Salinity–mineral nutrient relations in horticultural crops," Scientia Horticulturae, vol. 78, no. 1–4, pp. 127–157. https://doi.org/10.1016/S0304-4238(98)00192-7

Hashemi, K., R. Abbaspour, and A. Khandan. 2015. "Effect of licorice root extract on germination and growth of wheat under salt stress," Journal of Stress Physiol. Biochem., vol. 11, no. 2: pp. 94–99.

Kaviani R. and M. Saeidi, 2021. "The synergistic effect of licorice extract and biofertilizers on salt-stressed soybean," Iranian J. Plant Biol., vol. 10, no. 4: pp. 22–32.

Munns, R. and M. Tester. 2008. "Mechanisms of salinity tolerance," Ann. Rev. Plant Biol., vol. 59: pp. 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Moghaddam, M.B. and H. Javanmard. 2020. "Licorice extract improves physiological parameters of tomato under saline conditions," J. Plant Nutrit., vol. 43: no. 3, pp. 404–413.

Patil, A.H., M.D. Baig, and N.S. Kurrey. 2018. "Effect of salinity on physiological parameters in citrus rootstocks," Int. J. Chem. Stud., vol. 6: no. 5, pp. 120–124.

Sinha, L. and S. Biswas. 2014. "Glycyrrhiza glabra: a plant for the future," J. Pharm. Pharmacol., vol. 66: no. 12, pp. 1639–1651.

Shabani, F., R. Kaviani, and M. Saeidi. 2019. "Effect of licorice aqueous extract on salt-stressed maize (Zea mays L.)," J. Plant Physiol. Breed., vol. 9, no. 2, pp. 71–78.