Bio stimulatory Role of Licorice Root Extract and Garlic Extract in Enhancing Vegetative and Biochemical Characteristics of Two Citrus Species

Dhafer Hashem Jawad [1*], Sanaa Hasan Abdalakhwa [2], Samaa Jafer Kahdum [3]
[1, 3] Department of Horticulture and Landscape, College of Agriculture, Al-Qasim Green University, Babylon 51013, Iraq
[2] College of Basic Education, University of Babylon, Iraq

* Corresponding Author: **Dhafer Hashem Jawad**

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Abstract
This research was lead in the lath house of Agriculture faculty, University of Al-Qasim Green, during the 2025 season to appraise the effects paper spray of licorice (*Glycyrrhiza glabra* L.) root extract and soil appeals of garlic (*Allium sativum* L.) extract on vegetative growing and selected biochemical characters of due citrus species. pair citrus types, (Citrus aurantium L.) sour orange and (Citrus reticulata Blanco) mandarin, were included in the research. Typical horticultural performs, including soil cultivation, weeding and irrigation, were performed rendering to recommended guidelines. The experimentation involved twofold main factors which citrus species and plant extracts. Treatments consisted of garlic extract soil application at 5 g L^{-1} and licorice root extract foliar spraying at 10 g L^{-1}. Both treatment was applied three times. Two growth periods (spring and autumn) per season across The experiment was arranged in RCBD a Randomized Complete Block Design, and variances among treatments were assessed at a chance level of 0.05. Data were statistically analyzed using GENESTAT software. The results showed the Soil application of garlic extract significantly outperformed foliar application of licorice root extract in most vegetative and biochemical parameters during both seasons. Garlic extract treatment recorded the highest mean values for stem length (81.5 cm), stem diameter (4.95 mm), number of branches (14.52 branches $seedling^{-1}$), and total flavonoid content (1.535 mg g^{-1} dry weight). In contrast, licorice extract treatments resulted in comparatively lower values for these traits. However, foliar application of licorice root extract significantly enhanced leaf area, reaching 382.78 cm^2, whereas garlic extract recorded lower values for this trait. The regarding species response, sour orange exhibited a significantly greater stem diameter (4.88 mm), while mandarin significantly excelled in number of branches (14.60 branches $seedling^{-1}$) and leaf area (377.82 cm^2). The communication between citrus species and plant extracts exposed significant effects on furthermost traits. The mixture of sour orange with garlic extract produced the maximum stem diameter (5.83 mm) and stem length (82.6 cm). In the meantime, garlic extract mutual with mandarin caused in the highest branches number (16.87 branches $seedling^{-1}$) and leaves number (136.3 leaves $seedling^{-1}$). Contrariwise, extract of licorice root united with sour orange significantly improved leaf area (458.72 cm^2). for the interaction between licorice extract and mandarin in leaf chlorophyll content No significant lead was practical, which reached 64.8 SPAD units. General, the results indicate that extract of garlic, chiefly when applied to soil, a prominent role plays in ornamental growth vegetativtion and traits certain biochemical in citrus, whereas extract of licorice root is extra effective in refining leaf area under the conditions.

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Introduction
The genus Citrus is in the family Rutaceae, which is among the most economically significant fruit crop families globally. They are mostly found in tropical and subtropical areas, with a latitude ranging 40° north and south of the equator and their origin is in Southeast Asia. Production Citrus is the second most produced crop in the world only after grapes and has high nutritional value, especially because of its abundance of vitamin C, moderate levels of vitamin A, B1 and B2, as well as essential minerals

like calcium, potassium, phosphorus and iron. Citrus farming plays an important role in the agricultural industry of Iraq, with millions of trees playing a big role in its national production. Citrus aurantium L. sour orange is a popular rootstock in the horticultural industry because it has a robust root system, adapts to diverse soil types and possesses resistance to some diseases. Mandarin (Citrus reticulata L.), however, is considered a good source of sweet, low-acidity fruits and has economic significance in fresh consumption and food processing business. The growing interest in the environmental and health effects of synthetic fertilizers and growth regulators has promoted the adoption of natural plant extracts as natural and eco-friendly alternatives. Garlic extract is endowed with high levels of sulfur containing compounds, amino acids, vitamins and natural antimicrobial compounds, which contribute to growth and productivity in plants. Likewise, licorice root extract has glycyrrhizin, flavonoid and growth-promoting compounds which have hormone like action especially similar to gibberellins thus increasing cell division, elongation and the overall growth of the plant.The purpose of the study was to assess how two citrus species would respond to the foliar and soil application of natural plant extracts, which are licorice root extract and garlic extract, and to assess their impacts on vegetative and biochemical characteristics in a controlled environment.

Materials and Methods

The experiment took place inside lath house of the Department of Horticulture and Landscape Gardening, College of Agriculture, Al-Qasim Green University in the 2024 2025 growing season. A certified citrus nursery propagation nursery with the Ministry of Agriculture, in the Al-Hindiya District, Karbala Province, supplied the citrus seedlings. A sample of 54 uniform, six-month-old seedlings (27 sour orange, Citrus aurantium L., and 27 mandarin, Citrus reticulata Blanco) was chosen. Plastic bags (2 kg capacity) were used to grow the seedlings and kept under lath house conditions. During the experimental period, all the conventional horticultural activities, such as irrigation, weeding, loosening of soils and pest management were done. Secondly, a recommended basal fertilization was done on all seedlings using NPK fertilizer. The preparation of plant extracts was done using locally sourced materials. The preparation of licorice (Glycyrrhiza glabra L.) root extract was done by grinding 10 g of roots (dried), soaking in 1 L of distilled water, mixing and filtering through muslin cloth as per the procedure given by Al-Marsoumi (1999). Garlic (Allium sativum L.) extract was made by homogenizing 5 g of fresh cloves in 1 L of distilled water and after 24 hours the extract was filtered through a muslin cloth as per Al-Amri (2001). The experiment was set up in the form of a Randomized Complete Block Design (RCBD) having three replicates. Three seedlings comprised each experimental unit, and thus 18 seedlings per replicate. Treatments were foliar application of licorice root extract 10 g L -1 and soil application of garlic extract 5 g L -1. The statistical analysis was conducted with the help of GenStat software and the means of the treatments were compared with the help of Least Significant Difference (LSD) test with the probability level at 0.05. Three times of application were applied per season separated by 10 days between consecutive applications. Applications were done on November 8, November 28, and December 8, 2024, in the fall and on February 25, March 5, and March 15, 2025, in the spring. One week after foliar spraying, soil application of garlic extract was done. Early morning spraying was done until foliage was entirely moistened. Some 0.5 m spacing between treatments was employed and physical barriers were employed to curb spray drift. The licorice extract solution was then mixed with a surfactant (Zahi) and 100 mL of garlic extract was put on each seedling at each application of the soil.

Studied Traits

Vegetative characteristics

15 days following the last application, measurements were taken and they included:

1. **Stem length (cm):** This is a measurement of the length of the main stem between the crown and the apex of the main stem taken with a measuring tape.
2. **Stem diameter (mm):** The stem diameter at 5 cm above the soil surface measured with a vernier caliper.
3. **Branch count (branches seedling -1):** Counted by hand, and averaged by seedling.
4. **Number of leaves (leaves seedling -1):** The number of leaves per seedling was counted.
5. **Leaf area (cm 2):** Determined by using the formula: leaf area = length of maximum length x width of maximum width x 0.66, according to Chou G. J., and multiplied by the total number of leaves on the seedling.

Chemical Traits

1. Leaf chlorophyll content (SPAD): The SPAD chlorophyll meter measured (German origin) on randomly selected leaf.
    a. **Total phenolics and flavonoids:** Determination by colorimetry using the HCl reagent.
        - **Extraction:** by Gregorio *et al.* 2 g of dried leaf powder was mixed with 40 mL 80% ethanol (v/v) and incubated at 32C and 180 rpm in a shaking water bath over 72 h.
        - **Total phenolics (mg g -1 DW):** The Folin-Ciocalteu method was used as described by Singleton and Rossi and adapted by Gregorio *et al*. (2020). Absorbance was recorded at 765 nm and the results were reported as mg/kg (dry weight) using gallic acid as a standard.
        - **Total flavonoids (mg/kg -1 DW):** As calculated by Zhishen *et al*. The absorbance was measured at 510 nm and catechin was calibrated.

Results and Discussion

1. Stem Length (cm)

The increase in the stem length as shown in Table (1) was statistically significant when using foliar application of garlic extract. The maximum mean Stem length was 81.5 cm, and the lowest was 57.25 cm under the control treatment. In terms of the influence of species, the results showed that there were no significant differences in the length of stems of the plant types being studied. Conversely, the treatment-to-treatment interaction had a high level of effect. The sour orange (Citrus aurantium) extract combined with garlic extract yielded the greatest mean stem length of 82.6 cm, but the lowest stem length (55.2 cm) was recorded in mandarin (Citrus reticulata) in the control.

Table 1: Effect of foliar application of licorice root extract and soil application of garlic extract on stem length (cm)

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	55.2	59.3	57.25
A2 Licorice root extract	72.3	77.5	74.9
A3 Garlic extract	80.5	82.6	81.5
Average	69.3	73.1	
L. S. D *0.05*	B 5.93	A 7.27	

2. **Stem Diameter (mm)**

The findings in Table (2) indicate that there was a significant rise in the stem diameter with treatment by the use of the garlic extract. The mean stem diameter was greatest, at 4.95 mm, and least, at 3.58 mm, under the control treatment. In terms of varietal performance, sour orange (Citrus aurantium) had the highest mean stem diameter (4.88 mm), with a significant difference over the other treatments, whilst mandarin (Citrus reticulata) had the lowest mean stem diameter (3.32 mm). The interaction effect was also found significant. The sour orange and garlic extract combination gave the largest stem diameter (5.83 mm) and the lowest stem diameter (2.67 mm) was recorded in the mandarin with the control treatment.

Table 2: Effect of foliar application of licorice root extract and soil application of garlic extract on stem diameter (mm).

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	2.67	4.50	3.58
A2 Licorice root extract	2.97	4.30	3.63
A3 Garlic extract	4.07	5.83	4.95
Average	3.32	4.88	
L. S. D *0.05*	B 0.627	A 0.768	1.086

3. Number of Branches per Plant (branch·plant^{-1})

The findings in Table (3) suggest that the treatment of the garlic extract had a significant effect on the increase in the number of branches per plant. The maximum mean of the number of branches was 14.52 branches per plant as compared to the minimum of 11.60 branches per plant under the control treatment. Among the varietal differences, the highest mean number of branches was observed in mandarin (Citrus reticulata) which was significantly higher than all other treatments with mandarin recording a mean value of 14.60 branches per plant, and sour orange (Citrus aurantium) recorded the lowest mean value (11.49 branches per plant). The effect of interaction was also high. The garlic extract plus mandarin treatment had the most number of branches (16.87 branches per plant), and the lowest number of branches (10.67 branches per plant) was observed in the control treatment.

Table 3: Effect of foliar application of licorice root extract and soil application of garlic extract on the number of branches (branch·plant^{-1}).

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	12.53	10.67	11.60
A2 Licorice root extract	14.40	11.63	13.01
A3 Garlic extract	16.87	12.17	14.52
Average	14.60	11.49	
L. S. D *0.05*	B 1.972	2.415 A	3.416

4. Number of Leaves per Plant (leaf·plant^{-1})

Table (4) results show that use of licorice root extract and garlic extract did not have a significant impact on the average number of leaves per plant. Nevertheless, in terms of varietal performance, mandarin (Citrus reticulata) had the highest average number of leaves (121.6 leaves per plant) with a significant difference over the other treatments but sour orange (Citrus aurantium) had the lowest average number of leaves (85.9 leaves per plant). The interaction effect, on the other hand, was statistically significant. Garlic extract combined with mandarin had the greatest mean number of leaves (136.3 leaves per plant), and the lowest number of leaves (91.1 leaves per plant) was observed in sour orange in the control treatment.

Table 4: Effect of foliar application of licorice root extract and soil application of garlic extract on the number of leaves (leaf·plant^{-1}).

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	112.6	91.1	101.9
A2 Licorice root extract	115.9	88.9	102.4
A3 Garlic extract	136.3	77.7	107.0
Average	121.6	85.9	
L. S. D *0.05*	B 5.01	A 7.86	15.05

5. Leaf Area (cm²)

According to the findings in Table (5), the use of licorice (Glycyrrhiza glabra) root extract tremendously enhanced the leaf area with an average of 382.78 cm² as opposed to the lowest of 266.98 cm2 in the control treatment. In terms of the varietal performance, mandarin (Citrus reticulata) had the largest average leaf area (377.82 cm 2) followed by all other treatments, sour orange (Citrus aurantium) had the lowest average leaf area (270.40 cm 2). The effect of interaction was significant. The mixture of sour orange with licorice root extract resulted in the highest leaf area (458.27 cm 2) and the lowest leaf area (363.11 cm 2) was in the sour orange with the control treatment. The tabulated data also show that the differences between the treatments were significant at the 0.05 probability level where LSD values of both factor A (treatments) and factor B (varieties) were 3.972 and 6.416 respectively, which validates the reliability of the observed differences.

Table 5: Effect of foliar application of licorice root extract and soil application of garlic extract on leaf area (cm²).

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	368.76	363.63	366.18
A2 Licorice root extract	383.11	458.27	382.78
A3 Garlic extract	381.65	365.08	373.36
Average	377.82	370.40	
L. S. D *0.05*	B 3.972	A 3.972	6.416

6. Chlorophyll Content in Leaves (SPAD Units)

Table (6) shows that the use of the two extracts of licorice (Glycyrrhiza glabra) root extract and garlic extract did not have a significant impact on the leaf chlorophyll content (SPAD values). Equally, there was no statistically significant effect of the differences between the plant varieties or the treatments and plant varieties on the chlorophyll content implying that the property did not change drastically due to the experimental conditions.

Table 6: Effect of foliar application of licorice root extract and soil application of garlic extract on chlorophyll content (SPAD units).

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	55.2	51.8	53.5
A2 Licorice root extract	64.8	57.1	61.0
A3 Garlic extract	54.1	63.1	58.6
Average	58.0	57.3	
L. S. D *0.05*	B 9.65	A 11.82	16.72

7. Total Phenolic Content (mg·g⁻¹ Dry Weight)

Table (7) shows that there was no significant effect on total phenolic content by the use of both licorice (Glycyrrhiza glabra) root extract and garlic extract. But on the varietal performance, mandarin (Citrus reticulata) was found to have the best mean total phenolic content (1.531mg/g-1 dry weight) significantly higher than the other treatments, and sour orange (Citrus aurantium) had the least mean value. The treatment and variety interaction did not have significant effect on the total phenolic content and this showed that the differences observed were mainly due to the varietal variation as opposed to the treatment interactions.

Table 7: Effect of foliar application of licorice root extract and soil application of garlic extract on total phenolic content (mg·g⁻¹ dry weight).

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	1.443	1.453	1.487
A2 Licorice root extract	1.480	1.493	1.535
A3 Garlic extract	1.497	1.527	1.512
Average	1.531	1.491	
L. S. D *0.05*	B 0.1434	A 0.1434	0.2484

8. Total Flavonoid Content (mg·g⁻¹ Dry Weight)

The findings in Table (8) reveal that the overall flavonoid content increased significantly under treatment with garlic extract achieving a highest mean of 39.30 mg / g -1 dry weight and the lowest mean of 35.02 mg / g -1 dry weight under the control treatment. In terms of varietal performance, sour orange (Citrus aurantium) had the best total flavonoid content mean (98.95 mg/g-1 dry weight), with better performance compared with all other treatments, and mandarin (Citrus reticulata) had the lowest mean (38.75 mg/g-1 dry weight). The interaction effect too was significant. The mixture of mandarin and garlic extract gave the highest mean total flavonoid content (40.55 mg -1 dry weight) and the lowest (37.15 mg -1 dry weight) was recorded under the respective interaction treatment.

Table 8: Effect of foliar application of licorice root extract and soil application of garlic extract on total flavonoid content (mg·g⁻¹ dry weight).

A	Plant B		Average
	mandarin (*Citrus reticulata* Blanco) B2	sour orange (*Citrus aurantium* L.) B1	
A1 control	38.55	33.92	35.02
A2 Licorice root extract	37.15	38.89	39.23
A3 Garlic extract	40.55	38.05	39.30
Average	38.75	38.95	
L. S. D *0.05*	B 0.611	A 0.611	1.551

Conclusion

The results demonstrated that the application of garlic extract showed a significant superiority over licorice (*Glycyrrhiza glabra*) root extract in most vegetative and chemical characteristics, including stem length, stem diameter, number of branches, and total flavonoid content. In contrast, foliar application of licorice root extract recorded lower values for most of these traits. On the other hand, licorice root extract showed a significant advantage in increasing leaf area, whereas garlic extract treatment resulted in the lowest values for this parameter. Regarding varietal response, sour orange (*Citrus aurantium*) recorded the highest stem diameter, while mandarin (*Citrus reticulata*) showed significant superiority in number of branches and leaf area. As for the interaction effects among the studied factors, the combination of garlic extract with sour orange resulted in a significant improvement in stem length and stem diameter. Moreover, the communication between extract of garlic and mandarin formed the highest morals for branches and leaves number.

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