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## **The Study of the effect of foliar spraying with humic acid and OMEX \_20 and their interaction on some of chemical Characteristics of seedlings of domestic sour lemon *Citrus limon* L.**

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### **Abstract:**

The study was conducted in a shaded area covered with Green Saran Net at a private nursery in Thi Qar Governorate, during the spring growing season of 2025, to investigate the effect of foliar spray treatments with humic acid at concentrations of (0, 2, and 3 gm. L<sup>-1</sup>), and the nutrient solution BIO-20 at concentrations of (0, 100, and 200 ml. L<sup>-1</sup>) and their interactions on increasing the chlorophyll and carbohydrate content, and the nutrients (nitrogen, phosphorus, and potassium) in the leaves of local lemon seedlings. The study results showed a significant advantage of humic acid spray treatment at a concentration of (3 gm/L<sup>-1</sup>) in increasing the leaf content of (chlorophyll, carbohydrates, nitrogen, phosphorus, and potassium), with average increases of 50.2 SPAD, 6.346 mg.gm<sup>-1</sup> dry weight, 1.83, 0.519, and 1.48%, respectively. The results also showed a significant advantage of spraying with the OMEXBIO-20 nutrient solution at a concentration of 200 ml.L<sup>-1</sup> in increasing the leaf content of chlorophyll, carbohydrates, nitrogen, phosphorus, and potassium. The average SPAD content was 47.15, 5.135 mg.gm<sup>-1</sup> dry weight, 1.41, 0.463, and 1.43%, respectively. The interaction treatment (3 gm. L<sup>-1</sup> humic acid + 200 ml. L<sup>-1</sup>) also had a significant effect on increasing the leaf content of chlorophyll, carbohydrates, nitrogen, phosphorus, and potassium, as follows 56.82 SPAD, 6.948 mg. gm<sup>-1</sup> dry weight, 1.88, 0.551, and 1.51%, respectively.

**Keywords:** Locally grown lemon, humic acid, BIO-20, foliar feeding.

## **Introduction:**

The lemon tree (*Citrus limon* L.) belongs to the genus *Citrus*. It is part of the Rutaceae family and is one of the oldest known trees. Ancients used it in medicine, particularly as an antidote to poisons [1]. The local lemon tree is characterized by its large size and long, horizontally growing branches. New growth and flowers are sometimes pink or purple. The leaves are medium-sized, light green, and stipule-free. They are generally thornless at the base, but if present, they are small. The leaf margins are serrated, and the fruit is oval-shaped, yellow when ripe, and highly nutritious [2, 3]. Its fruits are distinguished by being rich in essential minerals for the human body, such as potassium, calcium, iron, magnesium, sodium, sulfur, and phosphorus. They are also a source of vitamin C, and significant amounts of vitamins A, B1, and B2 [4].

Lemons are used in juice making and fresh consumption. They are also used as a flavoring for many foods. Furthermore, they have a positive effect on strengthening the nerves and glands. They have a significant effect in treating rheumatism and cancer, and they increase urination. Therefore, they are an effective treatment for kidney diseases and a nerve tonic. They are also used to treat arteriosclerosis and high blood pressure [5].

Foliar fertilization is a modern and effective technique for nourishing plants, by spraying nutrients directly onto the leaves, this method helps the plant obtain up to 85% of its needs. It is ideal, especially for trees with deep

roots, which may have difficulty absorbing nutrients from the soil, it helps supplement soil nutrition [6].

The availability of healthy and vigorous citrus seedlings, one of the most important means of spreading and developing citrus cultivation in Iraq. This requires adequate care, especially fertilization. The seedlings use many nutrients from the soil for various biological processes. Therefore, they must be replenished with appropriate fertilization [7].

Foliar feeding with biofertilizers is considered a modern and sustainable technique. Foliar fertilization is used as a means to overcome nutrient deficiencies in plants [8]. Spraying lemon seedlings with nutrient solutions, leads to a significant increase in vegetative and chemical characteristics [9]. Therefore, spraying the plant's foliage with fertilizers, containing macro and micronutrients in various forms, stimulates plant growth and increases its yield [10, 11].

Humic acid is an organic acid that is naturally produced from humic compounds resulting from the decomposition of organic matter. This affects plant growth through its influence on physiological processes such as photosynthesis and respiration [12].

Organic matter constitutes approximately 80% of humic acid. This acid is characterized by its high molecular weight, exceeding 3500 Daltons, and its molecular diameter ranges from 1500 to 3000 Å [13]. It also plays a vital role in enhancing

plant growth and quality. This acid is highly soluble in alkaline solutions, while it precipitates in acidic solutions at a pH of approximately 2 [14].

Humic acid directly and indirectly affects plant growth and development, plays a role in stimulating enzymes in numerous biological processes, enhances the plant's resistance to biological stress, improves chlorophyll synthesis and the rate of photosynthesis, aids in the secretion of sugars and amino acids, it increases cell wall thickness [15]. Adding two levels of humic acid to one-year-old lemon seedlings, led to an increase in the chlorophyll and carbohydrate content of the leaves [16].

Therefore, this research aims to study the effect of the BIO-20 nutrient

solution, humic acid, and their interaction on increasing the chlorophyll, carbohydrate, and nutrient content of leaves. This will improve the growth of local lemon seedlings, and produce high-quality seedlings, to contribute to the improvement and development of citrus orchards in Iraq.

### Materials and Methods:

The study was conducted in a private nursery during the spring season of 2025 in soil with the characteristics shown in Table (1), according to Black [17]. Soil samples were taken from the growing soil of the seedlings. Soil analysis was performed to determine its properties. Saplings of uniform age and size were selected.

**Table (1) Some soil properties.**

Items	Unit	Value
pH	-	7.30
Ec	ds. m <sup>-1</sup>	1.50
Sand	gm. kg <sup>-1</sup> soil	805.00
Clay		84.00
Silt		106.00
Soil texture	Sandy loam	

The experimental components were: humic acid at concentrations of 0, 2, and 3 gm. L<sup>-1</sup>, and BIO-20 nutrient solution at concentrations of 0, 100, and 200 ml. L<sup>-1</sup>.

The experiment was conducted using a RCBD design with three replicates [18]. Two factors were used: the first included humic acid at concentrations of 0, 2, and 3 gm. L<sup>-1</sup>, and the second included the BIO-20 nutrient solution at concentrations of 0, 100, and 200 ml. L<sup>-1</sup>. Nine treatments were

performed with three replicates. The total number of experimental units was 27, with three seedlings per treatment per replicate. Therefore, the total number of seedlings was 81.

### Characteristics studied:

**Estimation of total leaf chlorophyll content:** Total chlorophyll was estimated using a chlorophyll meter, to estimate the intensity of direct chlorophyll pigment using a Spad-Plus CCM-200 meter, readings were taken from 10 fully extended leaves, from

the fifth to the tenth node, of each seedling in each experimental unit [19].

**Carbohydrate ratio:** The carbohydrate content was determined using the phenol and sulfuric acid method [20]. 0.5 gm of dry leaf material was taken after washing and drying, it was placed in a 250 mL flask and 75 mL of distilled water was added. The solution was placed in a water bath at 90°C for one hour. 5 mL of the filtrate was filtered and 25 mL of distilled water was added. 1 mL of the filtrate was taken for each treatment, and 1 mL of phenol and 5 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) were added. The solution was then spectrophotometrically analyzed at 490 nm. The following equation was used:

$$\text{Carbohydrate Percentage} = \frac{\text{Concentration} \times \text{Dilution}}{\text{Sample Weight}} \times 100$$

**Determination of Nitrogen, Phosphorus, and Potassium Content of Leaves:** A sample of fully grown leaves weighing 5.0 gm was taken and dried in an oven at 65°C for 48 hours. The samples were then ground and thoroughly mixed to homogenize them. They were digested with 4% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and perchloric acid (HClO<sub>4</sub>) according to Cresser and Parsons [21] method. The digestate was transferred to a 100 cm<sup>3</sup> flask. The volume was then filled with distilled water, and the nitrogen, phosphorus, and potassium (K) content was determined.

**Estimation of the nitrogen element (N):** The total nitrogen in the leaves

was estimated using the Micro-kjeldal device according to the Bremner method as stated in Page *et al.* [22].

**Estimation of the phosphorus element (P):** The percentage of phosphorus was estimated using a spectrophotometer at a wavelength of 700 nanometers according to Olsen *et al.* [23] method.

**Estimation of the element potassium (K):** The element potassium in the leaves was estimated by the flame photometer according to Page *et al.* [22] method.

#### **Statistical Analysis:**

The statistical analysis of the experimental data was performed using the Genstat 12 statistical software for analysis of variance. The Least Significant Differences (LSD) test was used, to compare the means of the treatments at a probability level of 0.05 [18].

#### **Results:**

##### **Leaf Chlorophyll Content:**

Statistical analysis revealed a significant effect of humic acid spraying, BIO-20 nutrient solution, and their interaction on total leaf chlorophyll concentration.

Table (2) shows that humic acid spraying significantly impacts leaf chlorophyll content. 3 gm. L<sup>-1</sup> treatment resulted in the highest chlorophyll content, reaching 50.2 SPAD units, surpassing the control H0 treatment, which yielded the lowest average of 38.22 SPAD units.

Conversely, 200 ml. L<sup>-1</sup> BIO-20 solution treatment resulted in the highest leaf chlorophyll content, reaching 47.15 SPAD units. Meanwhile, the H0 treatment yielded the lowest average, at 40.09 SPAD units.

The interaction of humic acid and BIO-20 solution also yielded positive

results. The treatment (3 gm. L<sup>-1</sup> × 200 ml. L<sup>-1</sup>) outperformed the other treatments, recording the highest average leaf chlorophyll content of 56.82 SPAD units. Conversely, the control treatment (0) yielded the lowest value for the same trait, at 35.87 SPAD units.

**Table (2) The effect of spraying with the nutrient solution, humic acid, and the interaction on chlorophyll concentration in the leaves of local lemon seedlings (SPAD).**

Humic acid (gm. L <sup>-1</sup> )	BIO-20 (ml. L <sup>-1</sup> )			Mean
	0	100	200	
0	35.87	39.43	39.43	38.22
2	40.63	40.83	45.20	42.22
3	43.77	50.01	56.82	50.2
Mean	40.09	43.07	47.15	
L.S.D <sub>0.05</sub>	H	B	H×B	
	1.251	1.351	2.702	

### Leaf Carbohydrate Content:

Statistical analysis revealed a significant effect of humic acid spraying, BIO-20 nutrient solution, and their interaction on the total carbohydrate content in leaves. Table (3) shows that humic acid spraying significantly impacts leaf carbohydrate content. 3 gm. L<sup>-1</sup> treatment resulted in the highest average carbohydrate content of 6.346 mg. gm<sup>-1</sup>, surpassing the control treatment, which yielded the lowest average of 3.331 mg. gm<sup>-1</sup>.

200 ml. L<sup>-1</sup> BIO-20 solution treatment resulted in the highest average leaf

carbohydrate content of 5.135 mg. gm<sup>-1</sup>. Conversely, the control treatment yielded the lowest average of 3.838 mg. gm<sup>-1</sup>.

The interaction of humic acid and BIO-20 solution also yielded positive results. The treatment (3 gm. L<sup>-1</sup> × 200 ml. L<sup>-1</sup>) outperformed the other treatments, recording the highest average leaf carbohydrate content of 6.948 mg. gm<sup>-1</sup>. Conversely, the control treatment (0) yielded the lowest value for the same trait, at 2.931 mg. gm<sup>-1</sup>.

**Table (3) Effect of spraying with nutrient solution, humic acid, and the interaction on the leaf soluble carbohydrate content of lemon seedlings (mg. gm<sup>-1</sup> dry weight).**

Humic acid (gm. L <sup>-1</sup> )	BIO-20 (ml. L <sup>-1</sup> )			Mean
	0	100	200	
0	2.931	3.411	3.651	3.331
2	3.147	4.471	4.807	4.141
3	5.433	6.657	6.948	6.346
Mean	3.838	4.846	5.135	
L.S.D <sub>0.05</sub>	H	B	H×B	
	0.331	0.328	0.659	

### Leaf Nitrogen Content:

Statistical analysis revealed a significant effect of humic acid spraying, BIO-20 nutrient solution, and their interaction on total leaf nitrogen content. Table (4) shows that humic acid spraying significantly impacts leaf nitrogen content. 3 gm. L<sup>-1</sup> treatment yielded the highest average nitrogen content at 1.83%, surpassing the control treatment H0, which produced the lowest average at 0.743%.

Conversely, 200 ml. L<sup>-1</sup> BIO-20 solution treatment resulted in the

highest average leaf nitrogen content at 1.41%, while the control treatment H0 yielded the lowest average at 1.18%.

The interaction between humic acid and BIO-20 solution also yielded positive results. The treatment (3 gm. L<sup>-1</sup> × 200 ml. L<sup>-1</sup>) outperformed the other treatments, recording the highest average leaf nitrogen content of 1.88%. Conversely, the control treatment (0) yielded the lowest value for the same trait at 0.64%.

**Table (4) Effect of spraying with nutrient solution, humic acid, and the interaction on the nitrogen content in leaves of local lemon seedlings (%).**

Humic acid (gm. L <sup>-1</sup> )	BIO-20 (ml. L <sup>-1</sup> )			Mean
	0	100	200	
0	0.64	0.75	0.84	0.743
2	1.12	1.45	1.53	1.36
3	1.78	1.83	1.88	1.31
Mean	1.18	1.34	1.41	
L.S.D <sub>0.05</sub>	H	B	H×B	
	0.041	0.041	0.082	

### Leaf Phosphorus Content:

Statistical analysis revealed a significant interaction between humic acid spraying and the BIO-20 nutrient solution on total leaf phosphorus

content. Table (5) shows that humic acid spraying significantly impacts leaf phosphorus content. 3 gm. L<sup>-1</sup> treatment yielded the highest average phosphorus content (0.519%), surpassing the control treatment (H0),

which produced the lowest average (0.355%).

200 ml. L<sup>-1</sup> BIO-20 solution resulted in the highest leaf phosphorus content (0.463%), while the control treatment (H0) yielded the lowest average (0.383%).

The interaction between humic acid and BIO-20 solution also yielded

**Table (5) Effect of spraying with nutrient solution, humic acid, and the interaction on the percentage of phosphorus in the leaves of local lemon seedlings (%).**

Humic acid (gm. L <sup>-1</sup> )	BIO-20 (ml. L <sup>-1</sup> )			Mean
	0	100	200	
0	0.302	0.362	0.403	0.355
2	0.361	0.398	0.436	0.398
3	0.486	0.521	0.551	0.519
Mean	0.383	0.427	0.463	
L.S.D <sub>0.05</sub>	H	B	H×B	
	0.0132	0.0132	0.0264	

#### Potassium Content in Leaves:

Statistical analysis revealed a significant interaction between humic acid spraying and the BIO-20 nutrient solution on total potassium content in leaves. Table (6) shows that humic acid spraying significantly affects potassium content in leaves. 3 gm. L<sup>-1</sup> treatment yielded the highest average content (1.48%), surpassing the control treatment (H0), which produced the lowest average (1.19%).

The treatment with BIO-20 solution at a concentration of 200 ml. L<sup>-1</sup> also

**Table (6) Effect of spraying with nutrient solution, humic acid, and the interaction on the potassium content in the leaves of local lemon seedlings (%).**

Humic acid (gm. L <sup>-1</sup> )	BIO-20 (ml. L <sup>-1</sup> )			Mean
	0	100	200	

positive results. The treatment (3 gm. L<sup>-1</sup> ×200 ml. L<sup>-1</sup>) outperformed the other treatments, recording the highest average leaf phosphorus content of 0.551%. Conversely, the control treatment (0) yielded the lowest value for the same trait at 0.302%.

resulted in the highest average potassium content in leaves (1.43%). Conversely, the control treatment (H0) produced the lowest average (1.23%).

The interaction between humic acid and BIO-20 solution also yielded positive results. The treatment (3 gm. L<sup>-1</sup> ×200 ml. L<sup>-1</sup>) outperformed the other treatments, recording the highest average leaf potassium content of 1.51%. Conversely, the control treatment (0) yielded the lowest value for the same trait at 0.89%.

<b>0</b>	0.89	1.32	1.38	1.19
<b>2</b>	1.33	1.36	1.40	1.36
<b>3</b>	1.47	1.48	1.51	1.48
<b>Mean</b>	1.23	1.38	1.43	
<b>L.S.D<sub>0.05</sub></b>	<b>H</b>	<b>B</b>	<b>H×B</b>	
	0.023	0.023	0.056	

### Discussion:

Tables 2, 3, 4, 5, and 6 show a significant improvement in the studied traits (chlorophyll, carbohydrates, and nutrients), when the plant is fed with humic acid at a concentration of 3 gm. L<sup>-1</sup>. This improvement can be attributed to the ability of humic acid to stimulate auxin activity in plant tissues, which play a crucial role in increasing cell division and expansion. This leads to an increase in vegetative volume, leaf branching, plant height, leaf area, and dry weight of the plant. The results obtained when spraying seedlings with humic acid were similar, with a significant increase in most chemical parameters. The results were similar to those obtained when spraying seedlings with the nutrient solution BIO-20, which achieved a significant increase in most vegetative indicators. This was also similar to the response of bitter orange seedlings to humic acid, and a significant increase in most traits. Chemical [24, 25, 26], this is consistent with Al-Hayani [16] and Al-Ismaili [27].

We also observe a significant improvement in the studied traits when treated with the BIO-20 nutrient

solution at a concentration of 200 ml. L<sup>-1</sup>. This is attributed to the solution's content of micro- and macro-nutrients. It also plays a role in activating the root system, increasing potassium and phosphorus absorption, and raising nitrogen levels in the leaves. This contributes to the formation of porphyrins, which are components of chlorophyll. Consequently, it increases photosynthetic products and the phosphorus content [28]. This contributes to the synthesis of amino acids, which are the building blocks of proteins and hormones, stimulates cell division and elongation, and increases pigment production, especially chlorophyll. This positively impacts carbohydrate levels, consistent with Al-Mousawi and Khudair [29] and Mohammed and Al-Sousou [30] and Fawzi *et al.* [31] findings.

### Conclusions:

The response of local lemon seedlings to spraying with 3 gm. L<sup>-1</sup> of humic acid was measured, considering all studied chemical properties and nutrient content in the leaves. Spraying the leaves with 200 ml of the nutrient solution per plant had a positive effect on all studied properties. The

interaction between humic acid and the nutrient solution significantly increased the leaf content of chlorophyll, carbohydrates, nitrogen, phosphorus, and potassium.

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