Effect of ground addition and foliar spraying of bio-fertilizer on some vegetative growth characteristics of local lemon (Citrus lemon L.) Saplings

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Abstract. This study was carried out in the lath house of the Department of Biology - College of Education for Pure Sciences – University of Anbar, during the autumn season (2020) with the aim of studying the effect of soil fertilization and foliar application of (Bio health) bio fertilizer on some of the vegetative growth characteristics of local lemon saplings. A factorial experiment with two-factors experiment was carried out according to the randomized complete block design (RCBD) , included 16 treatments, with four replicates, the first factor (A) was the soil fertilization with at four concentrations of 0, 2, 4 and 6 g.L-1, the second factor (B) was foliar application with at four concentrations 0, 1, 2 and 3 g.L-1, the results showed the superiority of the treatment of soil fertilization with a concentration of 6 g.L-1 and the foliar application of 3 g.L-1, and the interaction between them was significant in the characteristics of scion diameter, leaves content of relative chlorophyll, leaf area and leaves area, as for the treatment, Foliar application with a concentration of 2 g.L-1 and 3 g.L-1 showed a significant increase in the characteristic of plat height and rootstock diameter respectively, while the interaction between soil fertilization and foliar application of 2 g.L-1 showed the best result in the characteristic of plant height, while the interaction between the soil fertilization treatments 1 g.L-1 and foliar application at a concentration of 2 g.L-1 showed the best diameter of rootstock.

1. Introduction:–
Citrus fruits belong to the Rutaceae family, which are small to medium-sized evergreen trees, the most important genus in which is Citrus. Citrus has nutritional importance in addition to its simple proportions of carbohydrates, proteins, fats and vitamins, but the health and medicinal value is very high [5]. Citrus fruits are the most important fruit crop in tropical and subtropical countries [11]. It also has an important role in being the most sought-after product in the global market as organic products [8].

Chemical fertilizers are an important and indispensable element for supplying the plant with the nutrients necessary for its growth, and if the plant does not get enough of the nutrients, symptoms of deficiency will appear on it from poor growth and failure of the plant to complete its life cycle in vegetative and fruiting growth, or indirectly affecting preventing the absorption of elements others are needed by the plant [7]. Nitrogen and phosphate fertilization is considered one of the most important agricultural applications, but in the long term it increases the risks of environmental pollution of water, air and soil with the remains of toxic elements such as lead, cadmium and arsenic [19]. Chemical fertilizers also affect the pH of the soil. At certain pH or alkaline levels, the absorption of some fertilizer elements increases at the expense of other elements and thus some non-toxic elements become originally toxic to some plants, and chemical fertilizers cause serial environmental problems such as loss of soil nutrients and their degradation. And reduce beneficial microorganisms [24]. Pollution of groundwater and drinking
water with nitrogen fertilizers is one of the main causes of cancer of the pancreas, bladder and thyroid gland [27]. Organic fertilizers and Bio fertilization are a good alternative to mineral fertilizers due to their physical, chemical and biological properties in increasing the soil's water-holding capacity and increasing the absorption capacity of nutrients [10]. Increasing crop production efficiency, improving soil, increasing fruit quality, reducing costs, preserving natural resources, and improving fertilizer absorption efficiency [14]. Accordingly, organic fertilizers and biological fertilizers must be part of an integrated fertilization system with industrial fertilizers and in the recommended quantities [22]. It is considered more effective in stimulating plant activity and increasing the plant's ability to withstand unsuitable conditions [26]. It enhances soil biodiversity, which in turn improves continuous plant growth and is rich in different microbial groups such as fungi and bacteria [18]. Where the microorganisms provide the necessary nutrients for plant growth from the unattractive form to the form ready for absorption, especially the nutrients such as nitrogen, phosphorus and potassium [20]. Soil microbes, or additives, along with biological fertilizers play an important role in many ecosystem processes, as well as in nutrient cycling and balance, decomposition of organic matter, and promoting plant health and growth [17]. At the present time, the use of foliar nutrition has become an important method for adding trace elements, while the use of ground application of fertilizers is the basic method [3]. The study aims to know the best concentration of Bio Health fertilizer that can be added as soil fertilization and foliar application to increase the growth characteristics and the leaves content of the elements.

**Materials and methods :-**

This study was conducted in the lath house of the Department of Biology - College of Education for Pure Sciences – University of Anbar for the period from 1/4/2020 until 1/12/2020, to study the effect of soil fertilization and foliar application of Bio health on some growth characteristics and leaf content of some elements. 64 saplings of local lemon budded on the sour orange rootstock were selected at the age of two years uniform in their growth as much as possible and transferred to the anvils with a capacity of 10 kg filled with soil (river soil + peat moss) at a ratio of 1: 1, soil samples were taken for the purpose of conducting physical and chemical analyzes It must be approved before carrying out the experiment, and as showed in Table (1).

**Table (1) the physical and chemical characteristics of the soil used in the experiment**

| Adjective          | Value | unit         |
|--------------------|-------|--------------|
| pH                 | 7.8   | S.CM⁻¹      |
| E.C                | 1.44  | g. kg⁻¹     |
| Organic matter     | 11.7  | g. kg⁻¹     |
| % nitrogen         | 1.39  | g. kg⁻¹     |
| % phosphorous      | 0.28  | g. kg⁻¹     |
| % Potassium        | 1.02  | g. kg⁻¹     |
| Soil sand          | 80.8  | g. kg⁻¹     |
| Silt               | 14.5  | g. kg⁻¹     |
| Clay               | 5.2   | g. kg⁻¹     |
| Soil texture       | Sandy |             |
The analyzes were carried out at the Upper Euphrates Basin Development Center, Anbar University.

The experiment was carried out using the German-made biological fertilizer Bio Health, the contents of which are shown in Table No. (2)

| Table (2) The components of the Bio health fertilizer |
|-----------------------------------------------------|
| the ingredients | Percentage |
|-----------------|------------|
| Humic acid      | 75%        |
| Water           | 10%        |
| Algae           | 5%         |
| Trichodermo hairzinum | 5%     |
| Bacillus subtilus | 5%     |

The biological fertilizer treatments:

The soil fertilization method were done in four concentrations of bio health fertilizer (0,2,4 and 6 g.L\(^{-1}\) ), And the foliar application with same fertilizer in four concentrations (0,1,2 and 3 g.L\(^{-1}\) ).

The biological fertilizer was added with irrigation water on 22/4/2020 and repeated on 22/9/2020. As for the foliar application, it was added on 10/4/2020 and repeated on 10/9/2020 using a 3 liter hand sprinkler until full wetness with the addition of a wetting agent (liquid soap) at a concentration of 0.1% [7].

Thus, the experiment was two factors (4×4) and four replications, as the experiment contained 16 treatments within the design of Randomized Complete Block Design (R.C.B.D). The data were analyzed according to the statistical program (Genestat) and the arithmetic averages were compared according to the L.S.D test at a probability level of 0.05 [6].

1- Plant height (cm):-

The height of the plant was measured using the metric tape, as it was measured from the soil surface to the growing apex, and averages were calculated for each experimental unit by dividing the total plant height by its number.

2- Diameter of the Rootstock (mm):-

The diameter of the rootstock was measured using the electronic Vernier caliper at a height of 5 cm above the soil surface and the rate was calculated for each experimental unit.
3- Diameter of Scion (mm):-

The diameter of the main stem was measured using the electronic Vernier caliper, at a height of 2 cm from the budding union for each saplings, and the rate was calculated for each experimental unit.

4- Relative Leaves content of chlorophyll (SPAD unit):-

The relative content of chlorophyll (SPAD unit) in leaves was calculated using a (Chlorophyll meter) Model SPAD-PLUS 502 manufactured in Minolta company.

5- Leaf area (cm²):-

10 leaves were taken at the fifth node to the eighth node from the top of the shoots [23]. Dividing the total by 10, we get the average area of one leaf from the following equation:

\[ \text{Leaf area} = \frac{2}{3} \times \text{length} \times \text{width} \]  

6- Leaves area (cm².sapling⁻¹):-

The number of leaves was counted before the experiment was conducted in September of 2020 and it was counted at the end of the experiment in January of 2021 and the rate of increase in the number of leaves was calculated by calculating the average area of one leaf and calculating the number of leaves on each sapling, the leaf area of one sapling was obtained according to the following equation:

leaves area = number of leaves per sapling \times \text{average area of one leaf (cm}²\)

Results and discussion:-

1- Plant height (cm):-

Table (3) showed that there was a significant difference in the plant height values, as the treatment of foliar application with bio health at a concentration of 2 g.L⁻¹ was superior to giving the highest height in lemon saplings of 88.01 cm compared to the lowest height of 83.17 cm for the treatment of foliar application of biological fertilizer at concentration 1 g.L⁻¹ and the 0 g.L⁻¹ treatment is 83.43 cm. while the soil fertilization treatments did not affect significantly on this trait. As for the interaction between the two study factors, the results in the same table showed significant differences, as the treatment of soil fertilization with a concentration of 2 g.L⁻¹ were superior to giving the highest height of lemon saplings of 89.43 cm compared with the lowest height of the additional soil fertilization of 2 g.L⁻¹ and the treatment of foliar spray at a concentration of 1 g.L⁻¹, which was 78.60 cm.

Table (3) The effect of soil fertilization and foliar application of bio health fertilizer on plant height (cm) of local lemon saplings

| Foliar application | B0 | B1 | B2 | B3 |
|--------------------|----|----|----|----|
| Plant height (cm)  |    |    |    |    |
2- Rootstock diameter (mm):

Table (4) shows that there is a significant effect on the diameter of rootstock, as the treatment of foliar application with the Bio health fertilizer at a concentration of 3 g.L⁻¹ outperformed in giving the largest rootstock diameter which reached 8.784 mm compared to the minimum diameter of 7.984 mm for the 0 g.L⁻¹ treatment. While the soil fertilization treatments did not affect significantly on this trait. As for the interaction between the study factors, the results the same table showed a significant differences, where the treatment of soil fertilization with a concentration of 2 g.L⁻¹ and the treatment of foliar application with a concentration of 3 g.L⁻¹ were superior to giving the largest diameter of the rootstock, reaching 9.195 mm compared to the minimum diameter of the rootstock at A0XB0 treatment, which reached 7.715 mm.

| Soil fertilization | 0 g.L⁻¹ | 1 g.L⁻¹ | 2 g.L⁻¹ | 3 g.L⁻¹ | mean A |
|--------------------|---------|---------|---------|---------|--------|
| A0 0g.L⁻¹          | 78.90   | 87.90   | 87.75   | 83.63   | 84.54  |
| A1 2g.L⁻¹          | 88.35   | 78.60   | 89.43   | 82.85   | 84.81  |
| A2 4g.L⁻¹          | 85.08   | 82.10   | 87.85   | 87.35   | 85.59  |
| A3 6g.L⁻¹          | 81.38   | 84.08   | 87.03   | 86.08   | 84.64  |
| mean B             | 83.43   | 83.17   | 88.01   | 84.98   |        |

Table (4) The effect of soil fertilization and foliar application of Bio health fertilizer on rootstock diameter (mm) of local lemon saplings

| Foliar application | B0    | B1     | B2     | B3     | mean A |
|--------------------|-------|--------|--------|--------|--------|
| Soil fertilization | 0 g.L⁻¹ | 1 g.L⁻¹ | 2 g.L⁻¹ | 3 g.L⁻¹ |        |
| A0 0g.L⁻¹          | 7.715 | 8.517  | 8.757  | 8.302  | 8.323  |
| A1 2g.L⁻¹          | 8.250 | 7.855  | 8.952  | 9.195  | 8.563  |
| A2 4g.L⁻¹          | 8.125 | 8.362  | 8.777  | 8.862  | 8.532  |
| A3 6g.L⁻¹          | 7.845 | 8.185  | 8.425  | 8.777  | 8.308  |
| mean B             | 7.984 | 8.230  | 8.728  | 8.784  |        |

3-Stem Diameter (mm) :

Table (5) showed a significant effect of treatment with biological fertilizer on the rate of increase in scion diameter of lemon saplings, where soil fertilization
treatment at a concentration of 6 g.L⁻¹ exceeded the highest average increase in scion diameter, which amounted to 7.726 mm compared to the lowest average increase in scion diameter reached 7.008 mm when compared with the 0g.L⁻¹ treatment. The same table showed a significant superiority in the treatment of foliar application with biological fertilizer at a concentration of 3 g.L⁻¹, as the highest average increase in stem diameter was recorded, which was 7.874 mm compared to the concentration of 1 g.L⁻¹ and the 0 g.L⁻¹ treatment reached 7.345 mm and 7.293 mm, respectively. As for the interaction between soil fertilization at a concentration of 6 g.L⁻¹ and the treatment of foliar application at a concentration of 3 g.L⁻¹ to give the highest rate of increase in scion diameter of 8.812 mm compared to the lowest rate of scion diameter It was 6.737 mm at A0×B0 treatment.

Table (5) The effect of soil fertilization and foliar application of bio health fertilizer on scion diameter (mm) of local lemon saplings

| Soil fertilization | Foliar application | B0  | B1  | B2  | B3  | mean A |
|--------------------|--------------------|-----|-----|-----|-----|--------|
| A0                 | 0 g.L⁻¹            | 6.737 | 6.743 | 7.358 | 7.195 | 7.008 |
| A1                 | 2 g.L⁻¹            | 7.905 | 7.258 | 7.455 | 7.128 | 7.436 |
| A2                 | 4 g.L⁻¹            | 7.883 | 7.180 | 7.340 | 8.362 | 7.691 |
| A3                 | 6 g.L⁻¹            | 7.293 | 7.345 | 7.455 | 8.812 | 7.726 |
| mean B             |                    | 7.454 | 7.131 | 7.402 | 7.874 |        |
| L.S.D 0.05         | A 0.458           |      |      |      |      |        |
|                    | B 0.458           |      |      |      |      |        |
|                    | AB 0.916           |      |      |      |      |        |

4-Relative chlorophyll content (SPAD):-

Table (6) shows that there was a significant effect of the treatments with biological fertilizer on the rate of increase in the relative chlorophyll content in the leaves, as the soil fertilization treatment at a concentration of 6 g.L⁻¹ gave the highest average chlorophyll content of 47,586 (SPAD unite) compared to the 0 g.L⁻¹ treatment was 40,538 (SPAD unite). We also note a significant superiority. When foliar application with a concentration of 3 g.L⁻¹, the highest average of relative chlorophyll content was recorded, which reached 44,879 (SPAD unite) compared to the 0g.L⁻¹ treatment that gave 43,691 (SPAD). As for the interaction between soil fertilization treatment. At a concentration of 6 g.L⁻¹ and a 3 g.L⁻¹ of foliar application treatment gave the highest relative chlorophyll content was 47.765 (SPAD unite) compared to the A0×B0 treatment, which reached 40.102 (SPAD unite).

Table (6) The effect of soil fertilization and foliar application of Bio Health on the relative chlorophyll content in leaves (SPAD) of local lemon saplings

| Foliar application | B0  | B1  | B2  | B3  |
|--------------------|-----|-----|-----|-----|
|                    |     |     |     |     |

| L.S.D 0.05         | A 0.458 | B 0.458 | AB 0.916 |
|--------------------|----------|----------|----------|
|                    |          |          |          |
Soil fertilization | 0 g.L\(^{-1}\) | 1 g.L\(^{-1}\) | 2 g.L\(^{-1}\) | 3 g.L\(^{-1}\) | mean A
A0 | 40.102 | 40.265 | 41.038 | 40.747 | 40.538
A1 | 41.462 | 42.072 | 42.570 | 43.902 | 42.502
A2 | 45.770 | 44.680 | 47.457 | 47.105 | 46.253
A3 | 47.430 | 47.725 | 47.422 | 47.765 | 47.586

mean B | 43.691 | 43.685 | 44.622 | 44.879

L.S.D 0.05 | A 0.463 | B 0.463 | AB 0.925

5- Leaf area (cm\(^2\)):-

Table (7) showed that there was a significant effect of treatments with biological fertilizer on the rate of increase in the leaf area, as the soil fertilization treatment at a concentration of 6 g.L\(^{-1}\) gave the highest average for a leaf area of 19.958 cm\(^2\) compared to the 0 g.L\(^{-1}\) treatment of 16.241 cm\(^2\). We also notice a significant superiority, as the treatment of foliar application at a concentration of 3 g.L\(^{-1}\) gave the highest average leaf area of 19.643 cm\(^2\) compared to the 0 g.L\(^{-1}\) treatment of 17.815 cm\(^2\). As for the interaction between the study factors, the results in the same table showed a significant superiority as it gave the treatment of soil fertilization at a concentration of 6 g.L\(^{-1}\) and the treatment of foliar application 2 g.L\(^{-1}\) to give the highest average leaf area, which amounted to 20,223 cm\(^2\) compared to the A0×BO treatment, which gave 12,862 cm\(^2\).

Table (7) The effect of soil fertilization and foliar application of bio health fertilizer on average leaf area (cm\(^2\)) of local lemon saplings

| Foliar application | B0 | B1 | B2 | B3 | mean A
Soil fertilization | 0 g.L\(^{-1}\) | 1 g.L\(^{-1}\) | 2 g.L\(^{-1}\) | 3 g.L\(^{-1}\)
A0 | 12.862 | 14.261 | 18.625 | 19.214 | 16.241
A1 | 19.226 | 18.727 | 19.412 | 19.159 | 19.131
A2 | 19.495 | 20.130 | 19.634 | 20.094 | 19.838
A3 | 19.676 | 19.824 | 20.223 | 20.107 | 19.958
mean B | 17.815 | 18.236 | 19.473 | 19.643

L.S.D 0.05 | A 0.367 | B 0.367 | AB 0.735

6 – Leaves area (cm\(^2\).Sapling\(^{-1}\)):-
Table (8) showed that there was a significant effect of the treatments with bio fertilizer on the rate of increase in the leaves area of leaves, as the soil fertilization treatment at a concentration of 6 g.L\(^{-1}\) gave the highest average for the total area of leaves of 797.6 cm\(^2\) compared to the 0 g.L\(^{-1}\) treatment of 605.7 cm\(^2\). The same table showed a significant superiority in the treatment of foliar application at a concentration of 3 g.L\(^{-1}\), the highest average for the total area of leaves was 774.3 cm\(^2\) compared to the 0g.L\(^{-1}\) treatment, which was 675.5 cm\(^2\). As for the interaction between soil fertilization at a concentration of 6 g.L\(^{-1}\) and the treatment of foliar application 3 g.L\(^{-1}\) to give the highest average of total leaves area, which amounted to 823.9 cm\(^2\), compared with the A0×B0 treatment, which gave 425.5 cm\(^2\).

Table (8) The effect of soil fertilization and foliar application of Bio Health on the average of leaves area (cm\(^2\).sapling\(^{-1}\)) of local lemon saplings

| Soil fertilization | Foliar application | B0   | B1   | B2   | B3   | mean A |
|-------------------|--------------------|------|------|------|------|--------|
| A0     | 0 g.L\(^{-1}\)        | 425.5| 621.0| 671.9| 704.3| 605.7  |
| A1     | 2 g.L\(^{-1}\)        | 749.5| 669.3| 757.0| 791.0| 741.7  |
| A2     | 4 g.L\(^{-1}\)        | 724.2| 786.0| 766.3| 777.8| 775.1  |
| A3     | 6 g.L\(^{-1}\)        | 802.7| 811.7| 798.0| 823.9| 797.6  |
| mean B |                    | 675.5| 722.0| 748.3| 774.3|        |
| L.S.D 0.05 |                    | A 42.01 | B 42.01 | AB 84.01 |        |

**Conclusion:**

The reason for the above results may be due to the fact that the bio fertilizers work to stimulate the plant growth regulators that have a major role in some vital processes in the plant for the purpose of increasing the availability of the nutrients in the soil and then absorbing them by the plant and thus reflected positively in plant growth [13]. Also, bio fertilizers, with all their extracts and microorganisms, stimulate vegetative growth indicators in plants as a result of the secretion of compounds that chelate some nutrients, which leads to an increase in the availability of these elements in the soil and an increase in their content in the plant [1]. The reason may also be attributed to the humic acid, which is present in large quantities in the bio fertilizer, as it increases the permeability of cell membranes and absorption of nutrients and increases their movement through the root capillaries [16]. Humic acid increases the efficiency of photosynthesis, the manufacture of carbohydrates and proteins, and reduces the decomposition of amino acids resulting from stress, and thus these effects achieve an increase in all vegetative characteristics [21]. Humic acid also stimulates cell division,
increases the growth rate, develops the shoots and roots, and increases dry matter in plant tissues [25]. These results are consistent with the findings of [2], [4],[15] and [12].

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