Effect of rootstock and bio-fertilizers on some mineral concentrations in the leaves of Local Lemon (Citrus limon L.) transplants and available nutrients in the Media

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Abstract

The study was conducted in the Lath-house/Department of Horticulture and landscape design/College of Agriculture and Forestry/University of Mosul on budded local lemon (Citrus limon L.) at one year old, by using Randomized Complete Block Design (RCBD) with two factors, three replications and four transplants for each experimental unit. First factor was rootstock(sour orange and Citrumelo swingle), second factor was inoculation with Bacillus subtilis bacteria and Trichoderma harzianum fungi, in addition to control treatment (without inoculation), as the inoculation with the bacterial and fungus suspension was carried out twice a season according to the treatments, by adding 10 ml transplant⁻¹ in each addition, first was in the mid-March and the second was after three months of first treatment. Results indicated that the transplants budded on C. swingle gave the highest values of leaves concentration and soil availability of N, P and available Fe in the media, while the transplants budded on sour orange gave the highest values of leaves K and Fe concentrations. As for biological fertilizers the inoculation with T. harzianum gave the highest values of leaves N and K concentrations and availability of N and Fe in the media, while the inoculation with B. subtilis gave the highest values of other parameters (leaves concentrations of P and Fe and P availability in the media). The interaction between rootstock and bio-fertilizers significantly effected on all studied characteristics, the interaction between C. swingle and T. harzianum gave the highest values of leaves N and available N in the media, while the interaction between C. swingle and B. subtilis gave the highest values of leaves P and its availability in the media, as for leaves K, the interaction between sour orange and T. harzianum gave the highest values of it, while the interaction between sour orange and B. subtilis gave the highest values of leaves Fe concentration.

Key word: Citrus limon L., Rootstocks, Bio-fertilizer

1. Introduction

Lemon (Citrus limon L.), is one of the citrus species belonging to the family Rutaceae, Northeastern India and Southwestern China are the original habitat of this species [1]. It is one of the types of citrus fruits cultivated in Iraq for a long time, because of the availability of favorable conditions for its cultivation, In addition, fruits were rich in mineral salts which were necessary to build the human body, such as potassium, calcium, iron, magnesium, sodium, sulfur and phosphorous, and it is a good source of some vitamins like C, A, B1, B2, and B12 [2]. Most citrus species reproduce by budding or budding on different types of citrus rootstocks, as each of these rootstocks has its own distinct characteristics that differ from each other, and the choice of the rootstock is related to its suitable to environmental conditions (climate and soil) as well as the degree of its compatibility with the scion and its physiological effect on the growth and production of the budded varieties on it, as the ideal rootstock of citrus that is characterized by rapid growth and highly compatible with the species and varieties that will be budded on it and lives for a long time, its fruits are of good quality and contain many seeds with many vegetative embryos[3]. The rootstocks provide the budded trees with many advantages, as through the process of budding, the desired traits in the scion and rootstock are combined into one tree to benefit each from the other, the main propagation method for citrus trees is budding or budding mostly on seed rootstocks, farmers have been interested in selecting strong growing rootstocks because of their effect in providing seedlings with strong growth and adapted to the conditions of the region [4], also the rootstocks affects the availability of the nutrients in the soil and their concentration in the leaves [5]. [6] confirmed the concentration of some nutrients in the leaves of three varieties of mandarin (Okitsu, Clausellina and Silverhill) that budded on three citrus rootstocks (Sour orange, Troyer strange and Carrizo strange), they observed that varieties budded on Carrizo...
strange gave the highest concentrations of N and K in the leaves, while the varieties budded on Troyer strange gave the highest concentrations of P and Fe in the leaves. [7] showed that citrus rootstocks differ in the efficiency of their roots in absorbing the added component of phosphorus in the soil, they found that the leaves of Pêra sweet orange variety budded on Rangpur lime rootstock contained a higher concentration of P as compared with the same variety budded on Cleopatra mandarin rootstock, due to the efficiency of their roots in absorbing phosphorus from the soil. [8] studying the effect of seven citrus rootstocks: Tuzcu 31-31, Carrizo citrange, Troyer citrange, Smooth seville, Brazil bitter orange, Calamondin and Volkameriana on the concentration of some nutrients in the leaves of “Rio Red” grapefruit grown on them, they find that the highest concentrations of N, P and K were in the leaves of variety budded on Carrizo Strang rootstock as compared to the other rootstocks. [9] indicated in their study on some mineral concentrations in the leaves of Citrus lemon seedlings (Kagzi Kalan cultivar) budded on eight different rootstocks, that there were a difference in the concentration of nutrients in the leaves of the cultivar according to the type of rootstock, as they found that the highest concentration of N were in the leaves of the cultivar budded on Rough lemon and 4-RLC rootstock, as for the rootstock of the Sour orange, it gave the highest concentration of Cu and Zn in the leaves, while the other rootstocks were similar in their concentration of Cu. While the highest concentration of leaves Fe, were in the cultivar budded on Rough lemon, RLC-4 and Karn Khatta rootstocks, and the highest values of Mn were in seedlings budded on Rough lemon. [10] found that Pumelo transplants budded on two citrus rootstocks (Sour orange and Volka mariana), the seedlings that budded on Volka mariana gave the higher concentrations of N, P and K in the leaves as compared to the seedlings budded on Sour orange rootstock.

Bio-fertilizer is one of the important things in organic agriculture to regulate production, protect the environment, and produce crops free of pollutants, as the microbial inoculations supply the plant with its need of some nutrients and facilitate its absorption through its contribution in converting the unavailable of these elements into available form that is easy to absorb as well as providing them to some plant growth regulators, some of them also aerosol nitrogen fixation N2 through their symbiotic or symbiosis living with the host, which reduces the use of chemical fertilizers as well as protecting plants from some pathogens, which leads to reducing production costs, reducing environmental pollution and its impact on the environment and people. [11] studied the effect of treatment with a suspension containing Bacillus subtilis bacteria at a concentration of 10 ml.transplant⁻¹ of Citrus grandis budded on two citrus rootstocks, the results showed a significant increase in the available concentrations of N and P in the soil and the concentration of these two elements in addition to K in the leaves. [14] showed that treating Thompson grape seedlings with three different types of biological fertilizers (Biogen, Nitrobeine and Rhizobacterine) caused a significant increase in N, P and K concentrations in the leaves. Therefore and due to the lack of previous studies in Ninava governorate on the effect of rootstock and bio-fertilizers on the availability of some nutrients in the soil and its concentrations in the leaves of local lemon, we carried out this study.

2. Materials and methods

This study was conducted in the Lath-house / Department of Horticulture and Landscape Design /College of Agriculture and Forestry/University of Mosul in Ninava Governorate for the period from 1/3/2020 to 1/12/2020, to study the response of local lemon transplants budded on sour orange and Citrumelo swingle rootstocks and soil inoculation with B. subtilis bacteria and T. harzianum. Fungus. The selected transplants of lemon were planted in polyethylene plastic bags with a capacity of 3 kg of mixed soil, the transplants were transferred in 1/2/2020 to 8 kg plastic bags containing an agricultural medium consisting of river soil and peat moss at a ratio of 3: 1 respectively and analysis according to [22]. Results of cultivation media analysis shown in (Table 1).

Table 1. Some physical and chemical properties of the cultivation media.*

| Characteristics | Measurement unit | Valua |
|-----------------|------------------|-------|
| Electrical conductivity | (disysimns.m⁻¹) | 0.14 |
| pH | ........... | 7.12 |
| Organic matter | % | 3.4 |
| Clay | g. kg⁻¹ | 287 |
| Silt | g. kg⁻¹ | 412 |
| Sand | g. kg⁻¹ | 301 |
| Texture | ........... | Silty loam |
| Available nitrogen | mg. kg⁻¹ | 7.51 |
| Available Phosphorus | mg. kg⁻¹ | 3.24 |
The experiment carried out in a Randomized Complete Block Design (RCBD), including two factors with three replications, using four transplants for each experimental unit, first factor was rootstock, as we used two citrus rootstocks (Sour orange and Citrumelo swingle), second factor was inoculation with B. subtilis bacteria and T. harzianum fungus separately, in addition to control treatment (without inoculation), as the inoculation with the bacterial and fungus suspension was carried out twice a season according to the treatments, by adding 10 ml.transplant⁻¹ in each addition, first was in the mid-March and the second was after three months of first treatment, therefore the experiment was consisted 72 transplants (2 x 3 x 3 x 4 = 72 transplants).

On the 1st of August of 2020, full expended leaves were collected from the transplants for each experimental unit from the annual growth of the transplants, then washed with distilled water several times, dried and placed in an electric oven at a temperature of 70 °C until the stability of the weight (48 hours), then it was ground with an electric grinder and 200 mg of each sample were taken and digested in Pyrex flasks by adding 5 ml of concentrated sulfuric acid for 24 hours according to the method suggested by [15], after that 1 ml of concentrated perchloric acid was added to it and heated digestion flask until a clear solution is obtained, then the liquid was cooled down and the volume was completed to 50 ml with distilled water, after which the solution was filtered and the concentration of some nutrients in the digestion solution was estimated. N concentration (%) estimated by using Microkjeldahl as described by [16]. Phosphorous concentration (%) estimated by using ammonium molybdates and ascorbic acid with Spectrophotometer at a wave length of 882 nanometers, according to the method mentioned by [17]. Potassium by using flame-photometer according to the method proposed by [18], while iron was estimated by using atomic absorption according to the method mentioned by [17], and at the end of the season, the availability of N, P and Fe in the media (mg. Kg⁻¹) were estimated, according to the methods reported by [17]. Data were statistically analyzed with computer using Genstat program and the means were compared using Duncans multiple range test at a probability error of 0.05 [20].

### Results and discussion

Data in tables (2 and 3) cleared that the rootstocks had a significant effect on the concentration of P in the leaves only, as the highest concentration of this element was in the leaves of local lemon budded on Citrumelo swingle rootstock, which significantly dominated over the Sour orange rootstock. In relates to the concentration of available nutrients in the soil, Citrumelo swingle rootstock significantly dominated in the concentration of P and Fe elements over Sour orange rootstock, while the concentration of available N in the media was not significantly affected by the rootstocks. These results may be due to differences in the biological interactions and processes that take place in the Soil-Root Interface, the phenotype and features of root uptake, nutrient transport and distribution between roots and vegetative growth [21], as well as anatomical differences between root systems [22]. These results are consistent with the findings of [7-10].

The results also showed that the addition of biological fertilizers had a significant effect in all studied parameters, as the transplants treated with T. harzianum fungus gave the highest concentrations of N and K in the leaves as well as the available N and Fe in the soil, while the treatment with B. subtilis bacteria gave the highest concentrations of P and Fe in the leaves, as well as the available of P in the soil as compared to the control treatment. The reason for that may be due to the increase in the decomposition of organic matter in the media, especially peat moss (table 1) by microorganisms added to the media (T. harzianum and B. subtilis) and the release of its nutrients, which may increase its availability in the soil, and then absorb it by the plants and concentrate it in the leaves. [23] showed that increasing the concentration of the element in the soil solution leads to increase its absorption by the plants, and this is in line with what [24] stated, that biological fertilization leads to an improvement in the level of N and P in the plant as a result of fixing nitrogen and availability of phosphorous from the organic matter added to the soil, also, these bio-fertilizers may be improve the growth of transplants through the secretion of growth regulators, (auxins, gibberellins and cytokinins), which are positively reflected in the depth and spread of roots and thus increase the absorption of nutrients from the soil and their accumulation in the leaves [25]. The increase in nitrogen may be due to the ability of nitrogen-fixing bacteria which were live on the surface of plant roots, and the source of organic carbon for them may be through the root secretions of the medium [26], and this is in the line of data obtained by [10,14,27,28].

| Available Potassium (mg. kg⁻¹) | 16.01 |
| Available Iron (mg. kg⁻¹)       | 1.241 |
### Table 2. Effect of citrus rootstocks and bio-fertilizer on N, P, K and Fe in the leaves of local lemon (*Citrus limon* L.) transplants*.

| Citrus Rootstocks | Bio-fertilizer | Means |
|-------------------|----------------|-------|
|                   | Control | *Bacillus subtilis* | *Trichoderma harzianum* |       |
| **Nitrogen ( %)** |         |                  |                  |       |
| Sour orange        | c1.28   | bc1.62            | ab1.99            | a1.63 |
| *Citromelo swingle* | bc1.44 | abc1.68           | a2.19             | a1.77 |
| Means              | b1.36   | b1.65             | a2.09             |       |
| **Phosphorous ( %)** |         |                  |                  |       |
| Sour orange        | c0.107  | bc0.135           | bc0.119           | b0.120|
| *Citromelo swingle* | c0.103 | a0.183            | b0.144            | a0.143|
| Means              | c0.105  | a0.159            | b0.139            |       |
| **Potassium ( %)** |         |                  |                  |       |
| Sour orange        | b1.05   | ab1.14            | a1.33             | a1.17 |
| *Citromelo swingle* | b1.01  | ab1.19            | ab1.18            | a1.12 |
| Means              | b1.03   | a1.12             | a1.25             |       |
| **Iron ( mg.kg\(^{-1}\))** |         |                  |                  |       |
| Sour orange        | bc51.16 | a56.35            | ab52.51           | a53.34|
| *Citromelo swingle* | c48.17 | ab54.71           | ab54.82           | a52.57|
| Means              | b49.67  | a55.53            | a53.66            |       |

*Means of each factor and their interaction for each parameter followed by the same letter are not significantly different from each other according to Duncan’s multiple ranges test at 0.05 level.

### Table 3. Effect of rootstocks and bio-fertilizer on available N, P, and Fe in the media of local lemon (*Citrus limon* L.) transplants*.

| Citrus Rootstocks | Bio-fertilizer | Means |
|-------------------|----------------|-------|
|                   | Control | *Bacillus subtilis* | *Trichoderma harzianum* |       |
| **Nitrogen ( mg.kg\(^{-1}\))** |         |                  |                  |       |
| Sour orange        | d12.53  | b17.73           | cd14.50           | a14.92|
| *Citromelo swingle* | e8.90  | c15.33           | a22.77            | a15.67|
| Means              | c10.72  | b16.53           | a18.63            |       |
| **Phosphorous (mg.kg\(^{-1}\))** |         |                  |                  |       |
| Sour orange        | d3.08   | c3.51            | d3.12             | b3.24 |
Results showed that the interaction between rootstocks and bio-fertilizer was significantly influenced on all studied parameters, the highest concentration of $P$ in leaves and soil were obtained in the interaction between Citrumelo swingle rootstock and inoculation of $T. harzianum$, while the interaction between Citrumelo swingle rootstock and inoculation of $B. subtilis$ gave the highest concentration of $P$ in leaves and media, as for $K$, the interaction between Sour orange rootstock and inoculation of $T. harzianum$ was dominated over several treatments, while the interaction between Sour orange rootstock and inoculation of $B. subtilis$ was significantly dominated in leaves iron concentration over some other treatments. We concluded from this study that the bio-fertilization with $T. harzianum$ fungus or $B. subtilis$ bacteria significantly improved the availability of some nutrients in the soil which were positively reflected on its concentrations in the leaves of local lemon transplants budded on Citrumelo swingle and Sour orange rootstocks.

*Means of each factor and their interaction for each parameter followed by the same letter are not significantly different from each other according to Duncan's multiple ranges test at 0.05 level.

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| Citrumelo swingle | d3.03 | a4.72 | b3.77 | a3.84 |
|-------------------|-------|-------|-------|-------|
| Means             | c3.06 | a4.11 | b3.44 |       |

| Iron ( mg.kg$^{-1}$) |
|---------------------|
| Sour orange         |
| b1.233              |
| a1.477              |
| a1.470              |
| b1.393              |

| Citrumelo swingle |
|-------------------|
| a1.433            |
| a1.520            |
| a1.508            |

| Means             |
|--------------------|
| b1.333             |
| a1.498             |
| a1.520             |

The table above shows the results of the experiment with different rootstocks and bio-fertilizers. The concentration of iron in the leaves and soil is measured in mg kg$^{-1}$.
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