

Article

## Response of Three Citrus Rootstocks to Organic and Biological Fertilizers

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### Abstract

This study was conducted in a lath house, Dept of Hort. and Landscape, College of Agricultural Engineering Sciences, Univ. During the 2021 growing season, Baghdad will investigate the influence of organic and Biological fertilizers on three Citrus rootstocks' growth and leaf mineral content. The first factor is the addition of liquid organic fertilizers Vit-Org (O) at three levels without addition (O<sub>0</sub>), soil addition at 10 ml.L<sup>-1</sup> (O<sub>10</sub>) and soil addition at 20 ml.L<sup>-1</sup> (O<sub>20</sub>). The second factor is the addition of nitrogen-fixing bacteria without addition (N<sub>1</sub>), add 30 ml.Transplant<sup>-1</sup> of *Azotobacter chroococcum* (N<sub>2</sub>) and add 30 ml.Transplant<sup>-1</sup> of *Azospirillum brasileense* (N<sub>3</sub>). The third factor is three citrus rootstocks: sour orange (R<sub>1</sub>), Rangpur (R<sub>2</sub>), and C-35 Citrange (R<sub>3</sub>). Treatments were replicated three times (three transplants in the experimental unit) at split blocks design in an R.C.B.D. The number of transplants used was 243 transplants. The experimental results showed that the addition of Vit-Org liquid organic fertilizer had a significant effect in increasing vegetative growth characteristics and leaves mineral content, as treatment with a concentration of 20 ml.L<sup>-1</sup> (O<sub>20</sub>) was superior to increase in stem diameter of 2.99 mm and increase in leaves nitrogen content of 1.49%, phosphorous was 0.35%. Treatment with a concentration of 10 ml.L<sup>-1</sup> (O<sub>10</sub>) was superior in increased leaves number of 66.27 leaves: transplants<sup>-1</sup> and leaf area of 17.70 cm<sup>2</sup>. The addition of nitrogen-fixing bacteria, especially the addition of *Azospirillum brasileense* (N<sub>3</sub>), showed significant superiority in most vegetative growth traits; it gave the highest increase in leaves, the number of 65.28 leaves.transplant<sup>-1</sup>, highest leaves nitrogen content of 1.56%, phosphorous 0.32%. The addition of these bacteria did not significantly affect the increase in stem diameter and leaf area. Rootstocks varied among themselves in vegetative growth characteristics and leaf minerals content, as Rangpur rootstocks (R<sub>2</sub>) excelled in increasing the stem diameter of 2.32 mm and the leaves number of 83.90 leaves.transplant<sup>-1</sup>. In contrast, sour orange rootstock (R<sub>1</sub>) was superior in increased leaf area of 21.49 cm<sup>2</sup> and leaves phosphorus content of 0.30%. In contrast, C-35 rootstock (R<sub>3</sub>) outperformed in leaves nitrogen content by 1.44%. Twice and triple interactions between the study factors significantly affected all studied vegetative growth traits and leaf mineral content.

**Keywords:** Citrus Rootstocks, Organic, Biological Fertilizers

## Introduction

Citrus trees belong to the Rutaceae family, characterized by oil glands with an aromatic odor in most parts of the plant that distinguishes it from other types of fruits. Citrus is one of the most important of these genera, as it includes most of the economically important species and varieties of citrus because of its adaptation to a wide range of environmental conditions ranging from a hot, humid tropical climate and regions with a warm subtropical climate to cold regions<sup>1</sup>. Most historians and scientists believe that the original habitat of citrus is not known precisely and is likely to be in the tropical and subtropical regions of Southeast Asia, namely Western India, China, Indonesia, some parts of Burma and some parts of Southwest Asia<sup>2, 3</sup>. Citrus trees have a distinguished position among fruit trees due to the importance of their nutritional, economic, medical and aesthetic fruits, as they are rich in mineral elements, especially calcium, phosphorous, potassium, iron, manganese, chlorine, sodium, sulfur, copper and others, as well as containing a good amount of vitamins, especially vitamin C as well as vitamins A, B1, B2. Eating citrus fruits play an essential role in activating the work of enzymes within the human body and providing energy that promotes health, in addition to the fact that fruits are a good source of dietary fiber with a low percentage of proteins and a shallow content of fats<sup>4, 1</sup>.

The addition of mineral fertilizers, which secures the needs of any crop, is accompanied by several problems, including the loss of elements, especially nitrogen and phosphorus, leading to environmental pollution, biomass deterioration of bacteria and fungi of critical importance in improving soil physical and chemical properties and nutrients availability, which led to the search for means an environmentally friendly alternative, including a return to organic fertilizers and bio-fertilizers or following the concept of organic farming<sup>5</sup>. It is difficult to deny that organic farming is one of the most important modern practices in fruit production since organic fertilization is one of the important ways to supply plants with nutrients without any negative impact on the environment, and the increase from it does not lead to plant damage that occurs when fertilizing with mineral fertilizers in large quantities. The organic matter also has a role in plant growth and yield, whether added to the soil or foliar spray<sup>6</sup>. Several studies have been conducted on the role of liquid organic fertilizers in the growth of fruit transplants and trees.<sup>7</sup> found that using some organic fertilizers, one of them is Vit-org with two concentrations (15, 30 mg. L<sup>-1</sup>) to apricot trees increases leaves' nitrogen and phosphor content.<sup>8</sup> found that liquid organic fertilizer (Vit -org) at 30 and 60 ml.tree<sup>-1</sup> caused significant increases in stem diameter, leaves dry weight and leaves mineral content for "Hollywood" plum trees, especially at 60 ml.tree<sup>-1</sup>.

Scientists searched for alternative methods of chemical fertilizers that are safe for human health and do not cause environmental pollution. An alternative was to use biotechnology to solve these problems. Biotechnologies include any technology in which a living organism, or part of a living organism, is used or organic and inorganic products from a living organism<sup>9</sup>. Environmental protection organizations have paid much attention to biofertilizers due to their influential role in sustainable development and this fundamental importance in terms of the exchange between organic, chemical and biological fertilizers and their impact on the quality and productivity of crops<sup>10</sup>. Among the most important biotechnologies are additives of biological origin called bio-fertilizers. Bio-fertilizers are preparations that contain microorganisms capable of supplying plants with the nutrients they need from natural sources, thus reducing dependence on various

chemical fertilizers. Several studies have been conducted to determine the role of biofertilizers in the growth and leaf mineral content of fruit trees, <sup>11</sup>, found that the addition of bio-fertilizers to peach transplants gave the highest leaf area, increase in stem diameter and highest leaf nitrogen and phosphor content especially when added *Azotobacter chroococcum* + *Azospirillum brasilense* + *Bacillus megatherium* to the soil. Also, <sup>12</sup> found that the added *Azospirillum brasilense* + *Bacillus megatherium* gave the highest leaf number, stem diameter, shoot length, and leaf nitrogen and phosphor content when he studied hawthorn transplants.

## Materials And Methods

This study was conducted in a lath house, Dept of Hort. and Landscape, College of Agricultural Engineering Sciences, Univ. During the 2021 growing season, Baghdad will investigate the influence of organic and Biological fertilizers on three Citrus rootstocks' growth and leaf mineral content. The first factor is the addition of liquid organic fertilizers Vit-Org (O) at three levels without addition ( $O_0$ ), soil addition at  $10 \text{ ml.L}^{-1}$  ( $O_{10}$ ) and soil addition at  $20 \text{ ml.L}^{-1}$  ( $O_{20}$ ). The second factor is the addition of nitrogen-fixing bacteria without addition ( $N_1$ ), add  $30 \text{ ml.Transplant}^{-1}$  of *Azotobacter chroococcum* ( $N_2$ ) and add  $30 \text{ ml.Transplant}^{-1}$  of *Azospirillum brasilemse* ( $N_3$ ). The third factor is three citrus rootstocks: sour orange ( $R_1$ ), Rangpur ( $R_2$ ), and C-35 Citrange ( $R_3$ ). Treatments were replicated three times (three transplants in the experimental unit) at split blocks design in an R.C.B.D. Number of transplants used was 243 transplants. The following parameters were determined in the experimental season:

Leaf area ( $\text{cm}^2$ ): Five leaves were taken from the middle position of the shoot randomly and measured leaf area ( $\text{cm}^2$ ). They were using a Digimizer program on the Windows 7 operating system.

Leaves number.

Stem diameter increase (mm): Stem diameter was measured using a (Vernier) at the beginning and end of the experiment and calculating the difference between them.

Leaf Mineral Content: samples of ten leaves from middle shoots according to <sup>13</sup>. Leaves were washed with tap water, rinsed with distilled water, and then dried at  $70 \text{ }^\circ\text{C}$  until constant weight, ground and digested according to <sup>14</sup>. Nitrogen was estimated by the micro-Kjeldahl method of <sup>15</sup>. Phosphorus was estimated by using a spectrophotometer by <sup>16</sup>.

The obtained results were subjected to Analysis of variance according to <sup>17</sup> using L.S.D. 0.05 for comparing differences between various treatment means.

## Results

Effects of liquid organic fertilizers and nitrogen-fixing bacteria and their interaction on vegetative growth in three citrus rootstocks: Data concerning the effect of treatments on leaf area, leaf number and increase in stem diameter are listed in Tables (1, 2 and 3). The data cleared that liquid organic fertilizers (Vit-Org) at  $10 \text{ ml.L}^{-1}$  ( $O_{10}$ ) significantly increased in leaf area of  $17.70 \text{ cm}^2$  and leaves a number of  $66.27 \text{ leaves.plant}^{-1}$ , as treatment with a concentration of  $20 \text{ ml.L}^{-1}$  ( $O_{20}$ ) was superior to increase in stem diameter of  $2.99 \text{ mm}$ , while lower values of these traits were in  $O_0$  treatment. Tables (1, 2 and 3) also show that the addition of nitrogen-fixing bacteria, especially the addition of *Azospirillum brasilemse* ( $N_3$ ), showed significant superiority in increased leaves the number of  $65.28 \text{ leaves.transplant}^{-1}$ , while the addition of nitrogen-fixing bacteria did not significantly affect the increased stem diameter and leaf area. Rootstocks varied

among themselves in vegetative growth characteristics, as Rangpur rootstocks (R2) excelled in increasing stem diameter of 2.32 mm and increasing leaves number of 83.90 leaves.transplant<sup>-1</sup>. At the same time, sour orange rootstock (R1) was superior in a leaf area of 21.49 cm<sup>2</sup>. The interactions between liquid organic fertilizers and nitrogen-fixing bacteria significantly affected the leaf area, especially the interaction treatment (N<sub>3</sub>O<sub>10</sub>) of 18.55 cm<sup>2</sup>, while interaction treatment (N<sub>3</sub>O<sub>20</sub>) significantly affected leaf numbers of 68.74 leaves.transplant<sup>-1</sup>, while interaction treatment (N<sub>1</sub>O<sub>20</sub>) significantly affects a stem diameter of 2.99 mm. The interactions between liquid organic fertilizers and rootstocks were significantly affected, especially when interaction treatment (O<sub>10</sub>R<sub>1</sub>) gave 22.06 cm<sup>2</sup> as leaf area and interaction treatment (O<sub>10</sub>R<sub>2</sub>) gave 90.81 leaves. Transplant<sup>-1</sup> as leaves number and 3.46 mm as increased in stem diameter. As for the addition of nitrogen-fixing bacteria and their interaction with rootstocks, the data in Tables (1, 2 and 3) cleared a significant effect of these traits. Triple interactions between study factors had a significant effect on these traits.

nitrogen-fixing bacteria (N)	organic fertilizers (O)	Rootstocks (R)			N × O
		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
N <sub>1</sub>	O <sub>0</sub>	23.78	15.10	11.09	16.65
	O <sub>10</sub>	21.23	18.12	11.14	16.83
	O <sub>20</sub>	25.05	17.26	10.21	17.51
N <sub>2</sub>	O <sub>0</sub>	18.17	16.06	13.20	15.81
	O <sub>10</sub>	25.06	16.29	11.16	17.50
	O <sub>20</sub>	20.28	16.22	13.15	16.55
N <sub>3</sub>	O <sub>0</sub>	20.08	14.13	13.30	15.84
	O <sub>10</sub>	19.90	23.05	13.31	18.55
	O <sub>20</sub>	19.87	17.29	13.06	16.74
L.S.D. 0.05		1.98			1.09
N × R					N
N <sub>1</sub>		23.35	16.83	10.81	17.00
N <sub>2</sub>		21.17	16.19	12.50	16.62
N <sub>3</sub>		19.95	18.15	13.22	17.11
L.S.D. 0.05		1.31			N.S.
O × R					O
O <sub>0</sub>		20.67	15.10	12.53	16.10
O <sub>10</sub>		22.06	19.15	11.87	17.70
O <sub>20</sub>		21.74	16.92	12.14	16.93
L.S.D. 0.05		1.31			0.62
R		21.49	17.06	12.18	
L.S.D. 0.05		1.19			

**Table 1. Effects of liquid organic fertilizers and nitrogen-fixing bacteria and their interaction on leaf area (cm<sup>2</sup>) in three citrus rootstocks.**

		Rootstocks (R)			N × O
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nitrogen-fixing bacteria (N)	organic fertilizers (O)	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
N <sub>1</sub>	O <sub>0</sub>	80.00	61.11	29.11	56.74
	O <sub>10</sub>	75.00	85.22	38.22	66.15
	O <sub>20</sub>	64.00	89.11	26.22	59.78
N <sub>2</sub>	O <sub>0</sub>	69.22	89.00	37.11	65.11
	O <sub>10</sub>	67.22	94.00	42.11	67.78
	O <sub>20</sub>	73.11	82.22	29.00	61.44
N <sub>3</sub>	O <sub>0</sub>	65.22	78.22	43.22	62.22
	O <sub>10</sub>	73.22	93.22	28.22	64.89
	O <sub>20</sub>	83.00	83.00	40.22	68.74
L.S.D. 0.05		5.79			3.37
N × R					N
N <sub>1</sub>		73.00	78.48	31.19	60.89
N <sub>2</sub>		69.85	88.41	36.07	64.78
N <sub>3</sub>		73.81	84.81	37.22	65.28
L.S.D. 0.05		3.36			1.94
O × R					O
O <sub>0</sub>		71.48	76.11	36.48	61.36
O <sub>10</sub>		71.81	90.81	36.19	66.27
O <sub>20</sub>		73.37	84.78	31.81	63.32
L.S.D. 0.05		3.36			1.94
R		72.22	83.90	34.83	
L.S.D. 0.05		2.53			

**Table 2. Effects of liquid organic fertilizers and nitrogen-fixing bacteria and their interaction on leaves number (leaf.plant<sup>-1</sup>) in three citrus rootstocks.**

nitrogen-fixing bacteria (N)	organic fertilizers (O)	Rootstocks (R)			N × O
		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
N <sub>1</sub>	O <sub>0</sub>	1.60	2.95	1.91	2.15
	O <sub>10</sub>	1.93	3.08	2.47	2.49
	O <sub>20</sub>	2.70	3.28	3.10	3.02
N <sub>2</sub>	O <sub>0</sub>	0.71	3.69	2.91	2.44
	O <sub>10</sub>	2.33	3.23	2.84	2.80
	O <sub>20</sub>	3.04	3.79	3.02	2.28
N <sub>3</sub>	O <sub>0</sub>	3.53	3.21	2.67	3.14
	O <sub>10</sub>	1.13	4.06	2.86	2.68
	O <sub>20</sub>	2.48	2.60	2.90	2.66
L.S.D. 0.05		1.09			0.62
N × R					N

N <sub>1</sub>	2.07	3.10	2.49	2.56
N <sub>2</sub>	2.02	3.57	2.92	2.84
N <sub>3</sub>	2.38	3.29	2.81	2.82
L.S.D. 0.05	0.66			N.S.
O × R				O
O <sub>0</sub>	1.94	3.28	2.50	2.57
O <sub>10</sub>	1.79	3.46	2.72	2.66
O <sub>20</sub>	2.74	3.22	3.01	2.99
L.S.D. 0.05	0.66			0.36
R	2.16	3.32	2.74	
L.S.D. 0.05	0.55			

**Table 3. Effects of liquid organic fertilizers and nitrogen-fixing bacteria and their interaction on increased stem diameter (mm) in three citrus rootstocks.**

Effects of liquid organic fertilizers and nitrogen-fixing bacteria and their interaction on leaves nitrogen and phosphor content in three citrus rootstocks: The data in Tables (4 and 5) cleared that the addition of Vit-Org liquid organic fertilizer had a significant effect in increasing leaves nitrogen and phosphor content, as treatment with a concentration of 20 mL.L<sup>-1</sup> (O<sub>20</sub>) was superior to increase in leaves nitrogen content of 1.49 % and phosphor was 0.35%. The addition of nitrogen-fixing bacteria, especially the addition of *Azospirillum brasileense* (N<sub>3</sub>), showed significant superiority in leaves' nitrogen and phosphor content and gave the highest leaves nitrogen content of 1.56% and phosphor of 0.32%. Rootstocks varied among themselves in leaf minerals content, as sour orange rootstock (R<sub>1</sub>) was superior in increased leaves phosphors content of 0.30%, whereas C-35 rootstock (R<sub>3</sub>) outperformed in leaves nitrogen content of 1.44%. Twice and triple interactions between the study factors significantly affected leaves' nitrogen and phosphor content.

nitrogen-fixing bacteria (N)	organic fertilizers (O)	Rootstocks (R)			N × O
		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
N <sub>1</sub>	O <sub>0</sub>	1.24	1.24	1.26	1.25
	O <sub>10</sub>	1.26	1.27	1.30	1.28
	O <sub>20</sub>	1.31	1.34	1.32	1.32
N <sub>2</sub>	O <sub>0</sub>	1.33	1.36	1.36	1.35
	O <sub>10</sub>	1.43	1.41	1.45	1.43
	O <sub>20</sub>	1.51	1.55	1.52	1.52
N <sub>3</sub>	O <sub>0</sub>	1.46	1.49	1.50	1.48
	O <sub>10</sub>	1.55	1.56	1.59	1.57
	O <sub>20</sub>	1.60	1.63	1.65	1.62
L.S.D. 0.05		0.047			0.028
N × R					N
N <sub>1</sub>		1.27	1.28	1.29	1.28

N <sub>2</sub>	1.42	1.44	1.44	1.43
N <sub>3</sub>	1.53	1.56	1.58	1.56
L.S.D. 0.05	0.024			0.016
O × R				O
O <sub>0</sub>	1.34	1.36	1.37	1.36
O <sub>10</sub>	1.41	1.41	1.44	1.42
O <sub>20</sub>	1.47	1.50	1.50	1.49
L.S.D. 0.05	0.024			0.016
R	1.41	1.43	1.44	
L.S.D. 0.05	0.011			

**Table 4. Effects of liquid organic fertilizers and nitrogen-fixing bacteria and their interaction on leaves nitrogen content (%) in three citrus rootstocks.**

nitrogen-fixing bacteria (N)	organic fertilizers (O)	Rootstocks (R)			N × O
		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
N <sub>1</sub>	O <sub>0</sub>	0.22	0.21	0.24	0.22
	O <sub>10</sub>	0.27	0.25	0.25	0.25
	O <sub>20</sub>	0.33	0.31	0.33	0.32
N <sub>2</sub>	O <sub>0</sub>	0.24	0.23	0.24	0.24
	O <sub>10</sub>	0.29	0.28	0.29	0.29
	O <sub>20</sub>	0.36	0.33	0.35	0.35
N <sub>3</sub>	O <sub>0</sub>	0.27	0.25	0.26	0.26
	O <sub>10</sub>	0.31	0.30	0.30	0.31
	O <sub>20</sub>	0.40	0.39	0.39	0.39
L.S.D. 0.05		0.033			0.019
N × R				N	
N <sub>1</sub>		0.27	0.26	0.27	0.27
N <sub>2</sub>		0.30	0.28	0.29	0.29
N <sub>3</sub>		0.32	0.31	0.32	0.32
L.S.D. 0.05		0.018			0.011
O × R				O	
O <sub>0</sub>		0.24	0.23	0.24	0.24
O <sub>10</sub>		0.29	0.28	0.28	0.28
O <sub>20</sub>		0.36	0.34	0.36	0.35
L.S.D. 0.05		0.018			0.011
R		0.30	0.28	0.29	
L.S.D. 0.05		0.012			

**Table 5. Effects of liquid organic fertilizers and nitrogen-fixing bacteria and their interaction on leaves phosphor content (%) in three citrus rootstocks.**

## Discussion

The results show that liquid organic fertilizers addition has a positive effect on vegetative growth characteristics, and this increase may be attributed to the effect of organic fertilizers in improving soil chemical, biological and physical characteristics—physiological processes such as increasing efficiency of photosynthesis in leaves<sup>18</sup> and consequently increasing vegetative growth. The reason is also that this fertilizer contains most of the macro and micronutrients necessary for fruit transplant growth<sup>19</sup>. The results show that adding soil microorganisms has positively affected the number of leaves. This increase may be attributed to the effect of biofertilizers in improving soil biological and physical properties in addition to chemical properties, which resulted from the release of larger quantities of nutrients available for absorption by roots (Tables 4 and 5) and consequently affect physiological processes such as increasing efficiency of leaves photosynthesis<sup>20</sup> and increasing its products represented by carbohydrates and thus increasing leaves number of transplants. These results are in harmony with those obtained by<sup>21</sup> who worked on olive trees and<sup>12</sup> who worked on hawthorn transplants.

Moreover, the increase in leaf mineral content due to organic fertilizers application is attributed to its role in increasing soil organic matter, then improving soil composition and increasing the amount of available elements of the plant that absorb and increase its concentration in it<sup>22</sup>.

However, the reason for the increase in leaves' nitrogen and phosphorous content is that the addition of these organisms to soil led to an increase in the concentration of these elements in soil solution, then increasing its readiness and consequently increasing its absorption by transplants roots and increasing its transmission and then increasing concentration of these elements in leaves. Many researchers also confirmed that increasing the concentration of elements in soil solution increases its absorption by plant<sup>23</sup>. These results agree with those obtained by<sup>11</sup> on peach transplants and<sup>24</sup> on olive trees.

## Conclusions

Due to the lack of studies on the role of organic and bio-fertilizers in general and citrus trees in particular, as well as to move away from fertilizers and chemical growth regulators, this study aimed to know the effect of adding liquid organic fertilizer (Vit-org) and biofertilizer in growth and leaf mineral content of three citrus rootstocks.

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