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### Effect of spraying with nano, traditional nutrient solution, and cytokinin on some vegetative traits for orange seedlings grafted on rangpur (*Citrus aurantium* L.) rootstock

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#### Abstract

Present investigation was carried out at the Khaykan district, southeast of Babil province, during the period 1/9/2023 to 1/6/2024, on Six-month-old orange seedlings grafted on Rangpur rootstock. The aim of the study is to examine the effect of spraying with nano nutrient solutions at concentrations (0.1.2 g/L), traditional nutrient solutions at concentrations 1.g/L, and cytokinin at concentrations (0, 50, 100, 200 mg/L) and their interactions in the vegetative traits of seedlings. The results indicated that spraying seedlings with the single and combined study treatments led to a significant increase in seedling height, stem diameter, number of branches, number of leaves, leaf area, and average dry weight of the shoot, as treatment with the nano nutrient solution 1 gm/L with a cytokinin concentration of 200 mg/L was superior. By obtaining the highest rates for these characteristics compared to the control treatment.

**Keywords:** Citrus, cytokinin, nano fertilizer, rootstock

#### Introduction

Orange (*Citrus sinensis*. L) belong to genus Citrus, which belongs to Rutaceae family. Orange trees grown in tropical and subtropical regions approximately located between the latitudes of 35° north and 35° south. Because of the organoleptic characteristics and the high nutraceutical value (especially to high content of antioxidants), oranges fruit are particularly appreciated (Seminara *et al.*, 2023) [21]

Orange is the most citrus species cultivated in the world, orange's world production reaching 75.57 million tons in a harvested area of 9.93 million hectares (FAO, 2023).

Oranges are considered one of the most important fruit trees for local consumption in Iraq, as the number of trees is about (6,383,881) trees, and the quantity of production amounted about (142,717) tons, with an average production of one tree is approximately 22.4 kg. Baghdad province ranks first in number of trees and production. (CSO, 2021).

Grafting is one of the most successful methods of citrus propagation (Hussain *et al.*, 2017) [12]. Rangpur is one of the most widely used rootstocks for grafting oranges (Carvalho *et al.*, 2022) [4]. Rangpur is considered one of the rootstocks that resist thirst and high levels of salts in the soil (Shirin and Ihsan, 2023), withstands heavy soil conditions (Shanmukha *et al.*, 2023) [22], and It is resistant to gum disease caused by *Phytophthora citrophthora* (Azevedo *et al.*, 2006) [2]

Orange seedlings grafted on Rangpur rootstock are characterized by; slow growth (Ferrer *et al.*, 2022) [11], needs a long time to reach the fruiting stage (Kumar *et al.*, 2024) [13], which leads to increased production costs. To overcome such situations, methods are used that help increase or

accelerate the growth of seedlings, including a nutrient solution and cytokines. The nutrient solution (traditional and Nano) contains macro- and micro-nutrients that are considered determinants of plant growth and development (Kumar *et al.*, 2021; Parwiz and Monib, 2023) [14, 17]. Cytokines help increase cell division, branches, leaf area and the plant's efficiency in absorbing nutrients and thus increase its growth (Prasad, 2022; Sosnowski *et al.*, 2023) [18, 25].

The research aims, through the use of both cytokines and the nutrient solution, to improve vegetative and root growth, accelerate the growth of orange seedlings, make them reach the appropriate size, shorten the time needed to reach the fruiting stage, overcome the slow growth of seedlings, and determine the best concentration of the study factors.

#### Materials and Methods

Six-month-old orange seedlings grafted on Rangpur rootstock, planting in 7kg plastic pots, were selected with uniform growth as much as possible from a certified citrus production nursery belonging to the Iraqi Ministry of Agriculture / General Directorate of Horticulture and Forestry in the Holy Karbala Governorate / Hindiyah District, to study the effect of spraying with nano- and conventional fertilizer and cytokinin solution on the most important growth indicators for these seedlings.

The experiment was implemented following a randomized complete block design (R.C.B.D.) as a factorial experiment (4 × 4). Two factors consist of one concentration (1. g/L) of traditional fertilizer, three concentrations (0.1.2 g/L) of Nano-fertilizer and four concentrations (0, 50, 100, 200

mg/L) of cytokinin. The experiment included (16) treatments with three replicates, and the experimental unit included 5 seedlings, thus the number of seedlings used in the experiment was 420 seedlings. The results were analyzed using the Gen Stat program, and the averages were compared using the least significant difference (L.S.D) test at a probability level of 0.05.

**The following traits were measured**

- 1. Average seedling height (cm):** The height of the seedling was measured from the grafting area to the top of the seedling using a metric tape measure for all seedlings in the experimental unit, and the average was calculated for them.
- 2. Average stem diameter (mm):** The diameters of the seedlings were measured using an electronic Vernier caliper at a distance of 5 cm from the grafting area, and the average was calculated for each experimental unit.
- 3. Average number of total branches (branch/seedling):** The total number of lateral branches on the main stem of each seedling was counted and the average was extracted
- 4. Number of leaves (leaf/seedling):** All leaves on the seedling and for all plants in the experimental unit were counted and the average was calculated.
- 5. Leaf area of seedling (cm<sup>2</sup>/seedling):** The leaf area of the seedling was calculated by extracting the average leaf area by multiplying (maximum length x maximum width x 0.66), according to what was stated by Chou, G.J. 1966 [5]. Then, the total area of the seedling was

extracted by multiplying the average leaf area by the number of leaves and the average was extracted.

- 6. Average dry weight of shoots (gm. Seedling-1):** The vegetative parts (leaves, vegetative branches, and stem) were placed in perforated paper bags after being cut into small pieces and dried in an electric oven at a temperature of 70° until the weight was constant. They were weighed using a sensitive scale for each treatment, and the rate was extracted.

**Results and Discussion**

**1. Seedling height rate (cm)**

It is clear from the results of Table (1) that spraying seedlings with nano fertilizer led to a significant increase in the rate of seedling height, and the highest rate of height reached (64.32 cm) in seedlings treated with a concentration of 1 g/L of Nano fertilizer compared to the lowest height (53.41 cm) in the comparison treatment. Spraying seedlings with cytokinin also had a significant effect in increasing the rate of seedling height, which reached the highest range of height (62.55 cm) in seedlings treated with a concentration of 200 mg/L, compared to the lowest rate (57.16 cm) in comparison seedlings. As for the interaction between the concentrations of the nutrient solution and cytokinin it was significant, as the seedlings treated with a concentration of 1 g/L nano fertilizer with 200 mg/L of cytokinin excelled in obtaining the highest rate for this trait, which reached (68.09 cm) compared to the lowest rates (48.42 cm) in the comparison treatment.

**Table 1:** Effect of spraying the nutrient solution and cytokinin and their interaction on the seedling height rate/cm

| The average | Concentration of the nutrient solution in g/L |        |                                  |                            | Cytokinin concentration mg/L       |
|-------------|---|--------|----------------------------------|----------------------------|------------------------------------|
|             | 2 Nano  | 1 Nano | traditional                      | 0                          |                                    |
| 57.16       | 61.25   | 63.70  | 55.30                            | 48.42                      | 0                                  |
| 57.38       | 61.64   | 62.01  | 54.47                            | 51.42                      | 50                                 |
| 60.34       | 62.50   | 63.50  | 59.79                            | 55.60                      | 100                                |
| 62.55       | 63.39   | 68.09  | 60.52                            | 58.21                      | 200                                |
|             | 62.19   | 64.32  | 58.52                            | 53.41                      | The average                        |
|             | Interference concentration<br>7.646           |        | Cytokinin concentration<br>3.823 | Nutrient solution<br>3.823 | L.S.D at level<br>Probability 0.05 |

The reason for the increase in the growth rate of seedlings is due to the effect of the nutrient solution in filling the plant's need for mineral elements necessary for photosynthesis, respiration, and various metabolic processes, as the nutrient solution contains primary elements (NPK) and microelements (Mn, B, Cu, Zn, Fe) in quantities balanced in the process of cell division and elongation (El-Amary and El-Sayed, 2021) [7]. The reason for the increase in seedling height as a result of treating them with cytokinin may be attributed to its role in Building proteins and enzymes for the process of cell division and expansion, which led to an increase in osmotic pressure within them, and then absorbing an amount of water and nutrients, and this reflected positively in increasing plant growth indicators, including seedling height.

**2. Average diameter of the main Stem (mm)**

It has been found from the results presented in Table (2) that treatment with Nano fertilizer had a significant effect on the average stem diameter, the maximum average stem diameter when treated with 1 g/L nano fertilizer reached (10.39 mm), while in the comparison treatment it was about (7.48 mm). However, treating seedlings with cytokinin did not significantly affect the average stem diameter. Regarding the interaction between the nutrient solution and cytokinin, they had a significant effect on the average stem diameter. From the results, we notice that the concentration of 1 g/L with 200 mg/L cytokinin was superior (11.90 mm). The lowest rates were in the comparison treatment, as it reached (6.59 mm).

**Table 2:** Effect of spraying nutrient solution and cytokinin and their interaction on average stem diameter (mm)

| The average                         | Concentration of the nutrient solution in g/L |        |                            |      | Cytokinin concentration mg/L       |
|-------------------------------------|---|--------|----------------------------|------|------------------------------------|
|                                     | 2 Nano  | 1 Nano | Traditional                | 0    |                                    |
| 7.82                                | 8.03  | 8.87   | 7.79                       | 6.59 | 0                                  |
| 8.15                                | 8.05  | 8.99   | 8.36                       | 7.11 | 50                                 |
| 8.47                                | 8.74  | 10.80  | 7.92                       | 7.76 | 100                                |
| 8.74                                | 9.57  | 11.90  | 7.73                       | 7.47 | 200                                |
|                                     | 8.89  | 10.39  | 8.20                       | 7.48 | The average                        |
| Interference concentration<br>1.944 | Cytokinin concentration<br>n.s                |        | Nutrient solution<br>0.972 |      | L.S.D at level<br>Probability 0.05 |

The reason for the increase in stem diameter as a result of spraying the nutrient solution may be due to the important nutritional elements the solution contains, such as nitrogen, iron, and zinc, which are involved in many vital processes that occur in the plant, such as the formation of amino acids, proteins, and enzymes that encourage increased cell divisions and cell elongation. Tissue growth increases, which leads to an increase in the activity of the cambium layer, which when divided gives this increase in diameter (Wloch *et al.*, 2023).

**3. Average number of branches (branch/seedling)**

We notice from the results of Table (3) that spraying with the nutrient solution has a significant effect on the average number of branches, as the 1 g/L nano fertilizer treatment

outperformed by giving it the highest rate of (6.86 branches/seedling), while the comparison treatment gave the lowest rate of (5.45 branches/seedling). Spraying with cytokinin also had a significant effect on the average number of branches, as it reached the highest rate when treated with a concentration of 200. mg/L to (9.01 shoots/seedling), while the comparison treatment gave the lowest rate for this trait, amounting to (4.75 shoots/seedling). It is noted that the interaction of study factors had a significant effect on this characteristic, and the highest rate of number of branches was reached when using 1 g/L nano fertilizer with 200 mg/L of cytokinin, and it reached (9.89 branches/seedling) compared to the lowest rate (4.27 branches/plant) in the comparison treatment.

**Table 3:** Effect of spraying with nutrient solution, cytokinin and their interaction on the average number of branches.

| The average                         | Concentration of the nutrient solution in g/L |        |                            |      | Cytokinin concentration mg/L       |
|-------------------------------------|---|--------|----------------------------|------|------------------------------------|
|                                     | 2 Nano  | 1 Nano | Traditional                | 0    |                                    |
| 4.75                                | 5.15  | 5.27   | 4.33                       | 4.27 | 0                                  |
| 5.99                                | 6.11  | 6.57   | 6.17                       | 5.12 | 50                                 |
| 6.13                                | 6.42  | 6.73   | 6.01                       | 5.39 | 100                                |
| 8.01                                | 8.67  | 98.8   | 7.48                       | 7.03 | 200                                |
|                                     | 6.58  | 6.86   | 5.99                       | 5.45 | The average                        |
| Interference concentration<br>2.669 | Cytokinin concentration<br>1.384              |        | Nutrient solution<br>1.384 |      | L.S.D at level<br>Probability 0.05 |

The reason for the increase in the number of main branches as a result of using the nutrient solution may be attributed to its role because it contains many nutrients that have a positive effect on the vital processes within the plant and increase the process of photosynthesis, which is reflected in the increase in the number of branches (Fathi, 2022) <sup>[9]</sup>. The reason for the increased number of branches in seedlings treated with cytokinin is due to its role in increasing division, expansion, and increasing the number of branches (Sharma *et al.*, 2022; Wu *et al.*, 2021) <sup>[23, 29]</sup>.

**4. Average number of leaves (leaf/seedling)**

The results of Table (4) indicate that the treatment with the nutrient solution significantly affected the average number of leaves, as the 1 gm/liter nano fertilizer treatment was superior in giving it the highest rate of (64.01

leaves/seedling), while the comparison treatment gave the lowest rate of (50.02 leaves/seedling).

It is also noted from the same table that treating seedlings with cytokinin has a significant effect on the average number of leaves, as the highest rate reached (66.43 leaves/seedling) when treated with a concentration of 200 mg/liter, while the comparison treatment gave the lowest rate for this trait (47.67 leaves/seedling). As for the results of the interaction between.

The study factors had a significant effect on this trait, as the seedlings treated with a concentration of 1 g/L of nano fertilizer and a concentration of 200 mg/L of cytokinin excelled by obtaining the highest average number of leaves (76.37 leaves/seedling) compared to the lowest rates (37.18 leaves/seedling). In the comparative treatment.

**Table 4:** Effect of spraying with nutrient solution, cytokinin and their interaction on the average number of leaves per leaf/seedling.

| The average                         | Concentration of the nutrient solution in g/L |        |                           |       | Cytokinin concentration mg/L       |
|-------------------------------------|---|--------|---------------------------|-------|------------------------------------|
|                                     | 2 Nano  | 1 Nano | Traditional               | 0     |                                    |
| 47.67                               | 51.14   | 55.01  | 47.37                     | 37.18 | 0                                  |
| 57.20                               | 60.11   | 63.36  | 55.56                     | 49.80 | 50                                 |
| 58.58                               | 61.36   | 61.32  | 57.04                     | 54.63 | 100                                |
| 66.43                               | 66.5  | 76.37  | 64.43                     | 58.44 | 200                                |
|                                     | 59.77   | 64.01  | 56.10                     | 50.02 | The average                        |
| Interference concentration<br>19.48 | Cytokinin concentration<br>9.74               |        | Nutrient solution<br>9.74 |       | L.S.D at level<br>Probability 0.05 |

The reason for the increase in the number of leaves as a result of spraying the nutrient solution and cytokinin is due to the same reasons that explained the increase in the height of the seedlings, and the number of branches, as by increasing these characteristics, the number of leaves increases.

### 5- Leaf area (cm<sup>2</sup>)

The results in Table (5) confirm that treating seedlings with the nutrient solution had a significant effect on the average leaf area, as the seedlings treated with a concentration of 1 g/liter of nano fertilizer excelled by giving them the highest rate of (1601.70 cm<sup>2</sup>), while the comparison treatment gave

the lowest rate of (1235.01 cm<sup>2</sup>). Spraying seedlings with concentrations of cytokinin also increased the leaf area. By increasing the concentration, the largest leaf area was (1603.59 cm<sup>2</sup>) at the concentration of 200 mg/L, with a significant difference from the comparison treatment, which gave the lowest leaf area (1160.65 cm<sup>2</sup>). The interaction between the concentrations of the nutrient solution and the cytokinin concentrations gave a significant effect on the leaf area of the seedlings. The highest leaf area reached (1824.37 cm<sup>2</sup>) in the treatment with 1 g/L nano fertilizer with 200 mg/L cytokinin, compared to the lowest rate of 925.23 cm<sup>2</sup> in the comparison treatment.

**Table 5:** Effect of spraying with nutrient solution, cytokinin and their interaction on the average leaf area cm<sup>2</sup>.

| The average                         | Concentration of the nutrient solution in g/L |         |                           |         | Cytokinin concentration mg/L       |
|-------------------------------------|---|---------|---------------------------|---------|------------------------------------|
|                                     | 2 Nano  | 1 Nano  | Traditional               | 0       |                                    |
| 1160.65                             | 1173.05                                       | 1382.32 | 1162.01                   | 925.23  | 0                                  |
| 1405.40                             | 1446.43                                       | 1572.81 | 1327.83                   | 1274.54 | 50                                 |
| 1468.42                             | 1473.36                                       | 1627.32 | 1425.17                   | 1347.86 | 100                                |
| 1603.59                             | 1584.15                                       | 1824.37 | 1613.43                   | 1392.44 | 200                                |
|                                     | 1419.24                                       | 1601.70 | 1382.11                   | 1235.01 | The average                        |
| Interference concentration<br>13.61 | Cytokinin concentration<br>6.55               |         | Nutrient solution<br>6.55 |         | L.S.D at level<br>Probability 0.05 |

The increase in leaf area as a result of spraying with the nutrient solution, especially when spraying more than once, may be due to the nutrient solution containing a group of elements such as nitrogen, which is a necessary element for most of the vital processes that occur within the plant, as it contributes to increasing the rate of cell division and elongation, i.e. an increase. In their size and number, which results in increasing in leaf area (Taiz and Zeiger, 2002) [27]? Iron also has a role in increasing the leaf area because it is involved in the formation of cytochromes that are important in the process of photosynthesis and respiration, as it was found that 80% of the total iron is found in the chloroplasts. This shows its importance in the process Photosynthesis, through which food is manufactured and in turn increases the leaf area (Abou Seeda *et al.*, 2021; Rout and Sahoo, 2015) [1, 20].

Zinc also plays an important role in increasing this trait because it helps build the amino acid tryptophane, which is the basic substance for the synthesis of the natural hormone (IAA), which increases cell division and expansion, as evidenced by its deficiency in leaves being accompanied by a decrease in leaf area (Mosa *et al.*, 2021; Suganya *et al.*, 2020; Mona *et al.*, 2019; Fatma *et al.*, 2017) [16, 26, 10].

The role of cytokinin in increasing leaf area is also due to increasing leaf growth as a result of cell elongation and increasing the effectiveness of leaves in the photosynthesis process, thus increasing vegetative growth (Sosnowski *et al.*, 2023; Carnelos *et al.*, 2022) [3]. Or the reason may be attributed to the spraying treatments leading to an increase in vegetative growth, especially the number of leaves, in

addition to (seedling height, diameter, branch length, and number of branches), which was reflected. To increase the paper space.

### 6. Average dry weight of shoots (gm. Seedling<sup>-1</sup>)

It is clear from the results of Table (6) that the highest rate of dry weight of shoots (36 grams) was achieved in seedlings treated with a concentration of nano fertilizer of 1 grams/liter, and it differed significantly from the control treatment, whose seedlings produced the lowest rates, which is (23.41 grams).

Spraying seedlings with cytokinin also had a significant effect in increasing the average dry weight For the shoots, the concentration used increased until this rate reached its highest weight (32.12 grams) in the seedlings treated with a concentration of 200 mg/L, while the lowest rates were (27.68 grams) in the untreated seedlings. The results of the interaction between the nutrient solution and cytokinin confirmed the presence of a significant effect on this trait, as the seedlings treated with a concentration of 1 g/l nano fertilizer with the highest concentration of cytokinin excelled by giving them the highest dry weight rate (38.10 g) compared to the lowest rates (19.74 g) for dry weight in Untreated seedlings.

The reason for the increase in the dry weight of the shoots of seedlings treated with the nutrient solution and cytokinin is due to the increase in the height of the seedlings, the number of branches, the number of leaves, and the leaf area, which leads to an increase in this trait.

**Table 6:** Effect of spraying with the nutrient solution and cytokinin and their interaction on the average dry weight of the shoots/g

| The average                         | Concentration of the nutrient solution in g/L |        |                            |       | Cytokinin concentration mg/L       |
|-------------------------------------|---|--------|----------------------------|-------|------------------------------------|
|                                     | 2 Nano  | 1 Nano | Traditional                | 0     |                                    |
| 27.68                               | 32.81   | 34.56  | 23.61                      | 19.74 | 0                                  |
| 29.84                               | 28.36   | 37.19  | 27.85                      | 25.98 | 50                                 |
| 30.56                               | 32.46   | 37.68  | 28.97                      | 23.15 | 100                                |
| 32.12                               | 35.40   | 38.10  | 30.23                      | 24.77 | 200                                |
|                                     | 32.25   | 36.88  | 27.66                      | 23.41 | The average                        |
| Interference concentration<br>8.594 | Cytokinin concentration<br>4.297              |        | Nutrient solution<br>4.297 |       | L.S.D at level<br>Probability 0.05 |

### Conclusion and Recommendation

From the results obtained in the study, it is clear that spraying with both Nano nutrient solution (1.g/L) and cytokinin (200.mg/L) achieved a significant increase in all the vegetative traits studied. The interaction between Nano nutrient solution (1.g/L) and cytokinin (200.mg/L) had a positive effect on most vegetative traits. Therefore, the study recommends using Nano nutrient solution (1.g/L) because it has an effect on most of the studied traits and is the best from an economic standpoint.

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