The Effect of Foliar Spraying With Some Growth Stimulants on Improving Vegetative Growth and Mineral Content of Seedlings of Navel Orange and Blood Orange

Adib Jassem Abbas Al Ahbaby1 and Tahseen Khalifa Gharib Al-Ani2

1,2College of Agriculture, University of Tikrit, Iraq.

1Email: anytahseen300@gmail.com

Abstract

This study was carried out in lath house of the Department of Horticulture and Landscape Engineering - College of Agriculture - Tikrit University, during the season 2020-2021, as a factorial experiment by spraying with three levels of the growth regulator Brassinolide (BL) which are 0.00, 0.015, 0.025 Mg.L⁻¹ and spraying with nutrient solution PRO-SOL (M) at three concentrations of 0.00, 200, 400. Mg.L⁻¹ to determine the effect of the growth regulator Brassinolide and the nutrient solution PRO-SOL in increasing the chemical content of some nutrients for the two types of navel orange V₁ and blood orange V₂. The results showed that blood orange V₂ was significantly superior to the cultivar Navel V₁ in the leaf area amounted to 2991 cm², the percentage of dry matter in the leaves was 35.39% and the percentage of mineral elements in the leaves was %N, 1.619 %K, 1.37%P, 0.204. And the best concentration Of Brassinolide 0.025 Mg.L⁻¹ with a concentration of PRO-SOL 400 mg.L⁻¹ has increased the dry matter percentage, leaf area and chemical content of elements for the navel orange variety V₁, and the concentration of Brassinolide 0.015 Mg.L⁻¹ with a concentration of PRO-SOL 400 mg.L⁻¹ has increased the dry matter percentage, leaf area and chemical content of elements for blood orange variety V₂.

Keywords: Brassinolide, BRs, PRO-SO, Navel orange, Blood orange

1. Introduction

Citrus belongs to the family Rutaceae and contains the genera Poncirus, this genus includes the three-leafed orange, the other genus is Fortunella, which includes the kumquat (called Japanese orange or ornamental orange), and the genus Citrus, which is the most important of the three genera, which includes the basic groups of citrus [1]. One of the most prominent varieties of oranges (Citrus sinensis L) that has a high economic value in the world, which requires focusing the research direction on two varieties, the first is the navel orange variety, which arises from a genetic mutation that occurred in Brazil, while the other variety is blood orange, whose fruits are crimson-colored pulp containing bags. The juiciness contains on the anthocyanin pigment, which gives the fruits a red and pink color. The deterioration of the orange seedlings and the weakness in the characteristics of vegetative growth in the first years of cultivation, it needs special care [2]. This matter has increased the interest of many researchers to study its causes and find appropriate solutions for it. Its deterioration is reflected positively in increasing the growth of seedlings through the use of many plant growth regulators and nutrient solutions on the vegetative total for its great role in plant growth and one of the most important growth regulators (Brassinolide) [3,4], where it participates in the regulation of many vital activities of the plant cell, that Brassinolide is the most species effectiveness of a group of brassinosteroids (BRs) in terms of biological activity and works to increase the growth and resistance of plants to biotic and abiotic stresses [5,3]. Its use externally is described as being environmentally friendly [6], as well as the use of foliar feeding is an important and complementary aspect of ground fertilization in terms of the plant's need for nutrients and that these nutrients, especially Minor Elements, are subjected to a process of washing, volatilization, sedimentation and stabilization in the soil, where a large part of these elements [7]. Since the lack of nutrients has negative effects on the vital processes that take place inside the plant, so it became necessary to provide these elements by spraying them on the vegetative system to be absorbed by the plant tissues [8].

This study aims to use some growth stimulants to obtain fast-growing citrus seedlings and to increase their content of nutrients and thus the formation of fruits quickly. The effect of the growth regulator Brassinolide on two cultivars of navel orange and blood orange in increasing the vegetative characteristics, to test the effect of the interaction between the growth regulator Brassinolide and the nutrient PRO-SOL in
improving the nutritional status of the two cultivars. The aim of the study is to know the effect of spraying the growth regulator and the nutrient solution and the interaction between them in improving the nutritional status of the two types of navel orange and blood orange and its reflection on the characteristics of vegetative growth.

2. Materials and Methods

The experiment was carried out in the wooden canopy of the Department of Horticulture and Gardening Engineering - College of Agriculture - Tikrit University for the agricultural season 2020 and the seedlings were planted. Seedlings were quoted (3/15) in anvils(26X15)cm². Two cultivars of oranges were planted the first navel orange, the second blood orange seedlings, 54 seedling, two seedlings per experimental unit. The medium of cultivation was the river mixture, and the soil texture was sandy mixture. The first spray was in (4/7, 2020) and the second spray was conducted two weeks after the first spray. The date for taking the traits was at the end of August

1. The first factor of the class (V): (Navel orange V₁, blood orange V₂).
2. The second factor spraying with the growth regulator Barssinolid (BL): in three concentrations: 0.000 BL₀, 0.015 BL₁, 0.025 BL₂ Mg.L⁻¹.
3. Agent III PRO-SOL Nutrient Solution (M) PRO-SOL Nutrient Solution Spray Three Concentrations0.00M₀, 200M₁, 400M₂ Mg.L⁻¹.

Ingredients of the nutrient solution

- Total Nitrogen(N) 10%
- Ammoniacal Nitrogen 8.0%
- Nitrate Nitrogen 0.9%
- Urea Nitrogen 1.0%
- Total Phosphate(P₂O₅) 52%
- Total Potassium(K₂O) 10%
- Trace Elements (EDTA) ppm
  - Boron(B) 200
  - Chelated Copper 500
  - Chelated Iron 1000
  - Chelated Manganese 500
  - Chelated Zinc 500
  - Molybdenum 5

2.1 Total leaf area (cm²)

The leaf area of seedlings was calculated from the average leaf area x the number of total leaves of the seedling.

2.2 Percentage of dry matter in leaves (%)

4 papers were taken from the experimental unit from the area that is confined between the old and new leaves in the growing apex and in different directions of the plant, then weighed with a sensitive electric scale and placed in an electric oven at 65 °C until the weight is stable, then the dry matter was calculated according to the following equation: Percentage of dry matter of leaves = dry weight / wet weight x 100

2.3 Mineral content of leaves

Fresh old leaves with their petioles [9] which are in the phase of stability and relative stability of the elements [10], were taken to estimate the nutrients in leaves N, P, K in the laboratory of the College of Agriculture, Tikrit University, then the taken leaves were washed with water to remove dust It was dried in an air oven at a temperature of 65° C until the weight was stable. It was milled to pass through a sieve with holes diameter of 2.0 mm, and the wet digestion process was carried out using sulfuric and pyrochloric acid, as stated in [11], and then the elements were estimated as follows:

- **Nitrogen N%**: Nitrogen was estimated by Micro kjeldahl device [12].
- **Phosphorous P (%)**: The phosphorous was determined using ammonium molybdate and ascorbic acid using a UV-VIS Spectrophotometer model 80 D, at a wavelength of 410 nm [13].
- **Potassium K%**: Potassium was determined by Flame photometer [11].

The experiment used a randomized complete block design (R.C.B.D.). And with three factors, three replications and two seedlings in the experimental unit using the least significant difference test LSD between the treatments, at the probability level of 0.05[14]
3. Results and Discussion

3.1 Paper area (cm$^2$)

Results of Table (1) indicated that there was a significant difference in leaf area, where the variety V$_2$ outperformed blood orange, which amounted to 2991 (cm$^2$), over the variety V$_1$. As for the effect of spraying with Brassinolide, the treatment BL$_2$ gave the highest value, reaching 3044 (cm$^2$). Results also indicate the effect of spraying with the nutrient solution PRO-SOL, where the M$_2$ treatment was superior, giving the highest value of 3057 (cm$^2$). While the results of the binary interaction between the cultivar and Brassinolide showed a significant difference, where the treatment BL$_1$ V$_2$ excelled, reaching 2644 (cm$^2$). While results of the binary interaction between the cultivar and Brassinolide showed a significant difference, where the treatment BL$_1$V$_2$ excelled reaching 3455(cm$^2$). As for the binary interaction between the cultivar and the nutrient solution, it was found that the M$_2$ treatment outperformed the rest of the treatments for the two cultivars, as it reached 2473 and 3641 (cm$^2$), respectively. From the data it is clear that the binary interaction between Brassinolide and the nutrient solution PRO-SOL, where the treatment BL$_2$M$_2$ outperformed, which amounted to 3615 (cm$^2$). As for the triple interaction between the cultivar, Brassinolide and PRO-SOL, where the treatment V$_1$BL$_2$M$_2$ outperformed the rest of the treatments for the variety V$_1$ navel orange, as the leaf area reached 3078 (cm$^2$). Where the treatment V$_2$BL$_2$M$_2$ outperformed the rest of the treatments for the variety V$_1$ navel orange, as the leaf area reached 4658(cm$^2$). While the comparison treatment was less valuable for the two categories.

Table 1. Effect of variety, spraying with prasinoloid and nutrient solution (PRO-SOL) and interaction between factors on leaf area (cm$^2$).

| V  | BL  | M$_0$ | M$_1$ | M$_2$ | BLXV |
|----|-----|-------|-------|-------|------|
| V1 | BL$_0$ | 1.362 | 1.739 | 1.770 | 1.624 |
| V1 | BL$_2$ | 1.619 | 2.172 | 2.572 | 2.121 |
| V2 | BL$_1$ | 2.038 | 2.816 | 3.078 | 2.644 |
| V2 | BL$_0$ | 1.547 | 2.115 | 2.562 | 2.075 |
| V1 | BL$_1$ | 2.087 | 3.621 | 4.658 | 3.455 |
| V1 | BL$_2$ | 2.575 | 4.055 | 3.703 | 3.445 |
| Effect M | V$_1$ | 1.871 | 2.753 | 3.057 | Effect V |
| Effect M | V$_2$ | 2.070 | 3.264 | 3.641 | 2.991 |
| Effect BL | M$_0$ | 1.455 | 1.927 | 2.166 | 1.849 |
| Effect BL | M$_2$ | 1.853 | 2.896 | 3.615 | 2.788 |
| Effect BL | M$_2$ | 2.307 | 3.436 | 3.390 | 3.044 |
| LSD5% | V  | 2.070 | M  | BL×V | M×V | M×BL | M×BL×V |

3.2 Percentage of dry matter in leaves (%)

Results of Table (2) indicate the superiority of the blood orange variety V$_2$ in the percentage of dry matter in the leaves, which amounted to 35.39% over the variety V$_1$. Also, the results showed the effect of spraying with Brassinolide, as the treatment BL$_2$ gave the highest value, which amounted to 36.06%. The results also indicate the effect of spraying with the nutrient solution PRO-SOL, where the treatment M$_2$ excelled, as it gave the highest value of 35.92 (%), while the results of the binary interaction between the cultivar and Brassinolide showed a significant difference, where the treatment BL$_1$ V$_2$ excelled, which amounted to 37.09%. Between the cultivar and the nutrient solution, where the treatment V$_2$M$_2$ excelled in the percentage of dry matter in the leaves, which amounted to 37.01%. The results of the bilateral interaction between the Brassinolide and the nutrient solution PRO-SOL, where the treatment BL$_1$M$_2$ excelled in the percentage of dry matter in the leaves, which reached 36.90 (%). It is evident from the results of the triple interaction between the cultivar Brassinolide and the nutrient solution PRO-SOL, where the treatment V$_2$BL$_1$M$_2$ excelled, as the percentage of dry matter in the leaves of the blood variety was 40.72%.
Table 2. Effect of cultivar and spraying with Brassinolide and Nutrient Solution (PRO-SOL) and the interaction between factors on the percentage of dry matter in leaves (%).

| BLXV | V | BL | PRP-SOL | M0 | M1 | M2 | BLXV |
|------|---|-----|---------|----|----|----|------|
|      | V1| BL0 | 32.61   | 33.21| 34.18| 33.33|
|      |   | BL1 | 34.18   | 34.26| 33.09| 33.84|
|      |   | BL2 | 34.21   | 35.24| 37.23| 35.56|
|      |   | BL0 | 31.15   | 32.62| 33.77| 32.52|
|      | V2| BL0 | 33.81   | 36.74| 40.72| 37.09|
|      |   | BL1 | 34.84   | 38.31| 36.54| 36.56|
|      |   | BL2 | 34.84   | 38.31| 36.54| 36.56|
|      | Effect M| | 33.47   | 35.06| 35.92| 35.92|
|      | Effect V| | 33.66   | 34.24| 34.83| 34.25|
|      | MXV| | 33.27   | 35.89| 37.01| 35.39|
|      | Effect BL| | 31.88   | 32.92| 33.97| 32.92|
|      | MXBL| | 33.99   | 35.50| 36.90| 35.47|
|      |     | BL2 | 34.53   | 36.77| 36.88| 36.06|

LSD5%

Table 3. Effect of cultivar and spraying with Brassinolide and Nutrient Solution (PRO-SOL) and the interaction between factors on the percentage of nitrogen in leaves (%).

| BLXV | V | BL | PRP-SOL | M0 | M1 | M2 | BLXV |
|------|---|-----|---------|----|----|----|------|
|      | V1| BL0 | 1.18    | 1.60| 1.06| 1.18|
|      |   | BL1 | 1.60    | 1.72| 1.88| 1.55|
|      |   | BL2 | 1.03    | 2.02| 2.21| 1.75|
|      |   | BL0 | 0.97    | 1.04| 1.75| 1.26|
|      | V2| BL0 | 1.01    | 2.14| 2.31| 1.82|
|      |   | BL1 | 1.11    | 2.15| 2.09| 1.78|
|      |   | BL2 | 1.01    | 2.14| 2.31| 1.82|
|      | Effect M| | 1.021   | 1.68| 1.97| 1.72|
|      | Effect V| | 1.01    | 1.58| 1.90| 1.50|
|      | MXV| | 1.01    | 1.58| 1.90| 1.50|
|      | Effect BL| | 0.96    | 1.02| 1.68| 1.22|
|      | MXBL| | 1.04    | 1.93| 2.10| 1.69|
|      |     | BL2 | 1.07    | 2.08| 2.15| 1.77|

LSD5%

3.3 Percentage of mineral elements N.P.K in leaves %

Results of tables (3,4,5) show that there is a significant difference in the percentage of mineral elements in the leaves, where the variety V2 outperformed blood orange, which amounted to 1.62 N %, 0.20% P, 1.37 K % over the variety V1 navel orange. And also "the effect of spraying with Brassinolide, where the treatment BL2 was superior, which amounted to 1.77 N %, 0.21 % P, 1.52 K %. The results also indicate the effect of spraying with the PRO-SOL nutrient solution, where the M2 treatment was superior, as it gave the highest value of 1.97 N %, 0.22 % P, 1.50 K %. While the results of the binary interaction between the cultivar and Brassinolide showed a significant difference, where treatment BL1 V2 outperformed, which amounted to 1.82 N %, 1.58 K %, and treatment BL2 V1 outperformed it, which amounted to 0.22 % P. As for the binary interaction between the cultivar and the nutrient solution, it was found that there was a significant difference, where the treatment V2 M2 outperformed the rest of the treatments, as it amounted to 2.05 N %, P% 0.23, 1.56 K%. It is noted from the results of the binary interaction between the brasinolide and the nutrient solution PRO-SOL, where the treatment BL2 M2 outperformed the percentage of nitrogen in the leaves, which amounted to 2.15 N %, 1.69 K%, and the treatment BL1 M2 outperformed the percentage of phosphorous in the leaves, which amounted to 0.24% P. The triple interaction between the cultivar, Brassinolide and the PRO-SOL nutrient solution showed a significant difference, where the treatment V2 BL1 M2 outperformed, as it reached for blood type 2.31 N, 0.28% P, 1.95 K%. While the comparison treatment was the lowest for all treatments.
Table 4. Effect of cultivar and spraying with Brassinoloid and Nutrient Solution (PRO-SOL) and the interaction between factors on the percentage of phosphorous in leaves (%).

| V    | BL  | PRP-SOL | BLXV |
|------|-----|---------|------|
|      | V1  | M0      | M1   | M2   |
| BL0  | 0.16| 0.17    | 0.19 | 0.17 |
| BL1  | 0.18| 0.20    | 0.20 | 0.19 |
| BL2  | 0.19| 0.22    | 0.25 | 0.22 |
| BL3  | 0.17| 0.18    | 0.21 | 0.19 |
| V2   | M0  | 0.17    | 0.20 | 0.24 |
| V1   | 0.19| 0.24    | 0.20 | 0.21 |
| V2   | 0.19| 0.24    | 0.20 | 0.21 |

Effect V
- V1: 0.18 ± 0.20
- V2: 0.22 ± 0.22

Effect BL
- BL0: 0.17 ± 0.20
- BL1: 0.19 ± 0.24
- BL2: 0.19 ± 0.24

Effect MX
- MXV: 0.17 ± 0.20

LSD5%
- BL0 × V: 0.02 ± 0.02
- BL1 × V: 0.02 ± 0.02
- BL2 × V: 0.02 ± 0.02

Table 5. Effect of cultivar and spraying with Brassinoloid and Nutrient Solution (PRO-SOL) and the interaction between factors on the percentage of potassium in leaves (%).

| V    | BL  | PRP-SOL | BLXV |
|------|-----|---------|------|
|      | V1  | M0      | M1   | M2   |
| BL0  | 0.87| 0.91    | 1.31 | 1.03 |
| BL1  | 0.99| 1.13    | 1.25 | 1.12 |
| BL2  | 1.20| 1.51    | 1.75 | 1.49 |
| BL0  | 0.92| 0.93    | 1.09 | 0.98 |
| V2   | M0  | 1.18    | 1.61 | 1.95 |
| V1   | 1.32| 1.70    | 1.64 | 1.55 |
| V2   | 1.32| 1.70    | 1.64 | 1.55 |

Effect V
- V1: 1.02 ± 1.18
- V2: 1.14 ± 1.41

Effect BL
- BL0: 0.89 ± 0.92
- BL1: 1.08 ± 1.37
- BL2: 1.26 ± 1.61

Effect MX
- MXV: 1.08 ± 1.37

LSD5%
- BL0 × V: 0.04 ± 0.04
- BL1 × V: 0.04 ± 0.04

The superiority of the second class bloody orange Abussrh variety in the characteristics of vegetative growth and due to the nature of the trees will be comparable to the growth in the local orange trees as for the orange Abussrh, the nature of the growth will be near of the ground [2] that the cultivation of varieties is important for the diversity of production and knowledge. Measuring the effects of spraying with growth regulators, especially the effect of prasinolide on navel orange and blood orange, whereas, the significant increase in leaf area as a result of spraying with the growth regulator bracinolide (BL) is consistent with the results obtained by [15] which showed an increase in the average leaf area of apricot with the increase in the levels of BL used, and with [16] in grapes that obtained the highest leaf area. The increase in leaf area in orange seedlings may be attributed to spraying it with Bracinolide, as it increases the leaves’ content of natural plant hormones that encourage growth such as IAA, GA3, Zeatin, and reduces the leaves’ content of ABA [15]. Most of the physiological cell processes such as cell division and elongation, the biosynthesis of cell wall components, and the synthesis of DNA, RNA and multiple proteins [17]. Among these roles, BRs act to increase dry matter in leaves., There is a positive response for both varieties of orange. The increase in the percentage of nitrogen, phosphorous and potassium in the leaves when spraying with Bersilcinolide and the nutrient solution can be attributed to the role of both in stimulating the division and expansion of plant cells, which increases the rate of vegetative growth, perhaps due to its role in stimulating the growth of leaves due to increased division and expansion. It also stimulates the formation of some photosynthetic enzymes to the accumulation of
starch in the leaves [18] It also goes back to the role of the nutrients included in the composition of the nutrient solution in activating the work of enzymes, cell division, building proteins, and increasing the manufacture of nutrients that help in the growth of plant tissues and reflecting this positively in increasing the content of the elements [19] It is clear through the results of the study that there is an excellence of a bloody orange category on the classes of vegetative growth and chemical content and It is a triangular interference between factorsV_2BL,M_2,V_iBL,M_2 Showing significant increase in vegetable qualities and papers content of elements.

**Conclusion**

Cultivation of the bloody orange category is a successful planting. Spraying the growth regulator and nutrient solutions with higher concentrations from the input or use the same trees on orange trees or other plants seedlings.

**References**

[1] Al-Munisi, F. A. 1975. Citrus, the scientific basis for its cultivation. First Edition - New Publications House - Alexandria.
[2] Aliwa, J. I. (2014). Perennial fruit, citrus, citrus, Damietta University, Faculty of. Agriculture, Egypt.
[3] Choi, Y., H.T.Inoue, Fujioka, H. Saimoto. and A. Sakurai. (1993). Identification of brassinosteroid - like active substances in plant –cell cultures. Biosci. Biotech. Biochem. 57: 860-861.
[4] Saleh, H. M. (2000). Instructions for the use of foliar fertilizers. Ministry of Agriculture / General Authority for Agricultural Extension and Cooperation.
[5] AL-Khafaji, M. A. (2014). Plant growth regulators, their applications and horticultural uses., College of Agriculture, University of Baghdad, Ministry of Higher Education and Scientific Research. Al-Madar University Press
[6] Esposito,D, S. Komarnytsky, S. Shapses and I. Raskin. 2011. Anabolic effect of Plant brassinosteroids. FASEB J. 25(10): 3708-3719.
[7] Wernar,T., V. Motyka, M.strnad and T.Schmutling.(2000) Regulation of plant growth by cytokinin.max plnk Institute for plant Research,Cologne,Germany.
[8] Khalil, T, H, Saba Jawad, and Qais, J, A,Mawgod, (2005). Effect of bait cultivar and spraying with Prosol foliar fertilizer on growth of apricot seedlings (Prunus armeniacaL). Research extracted from a master's thesis.
[9] Mawhoub Amirouche, Dalila Smadhi, Lakhdar Zella and Hakim Bachir, 2021, Rainfall Trends in Semi-Arid Cereal Regions of Algeria, Al-Qadisiyah Journal For Agriculture Sciences, 11, 1, 36-44. doi: 10.33794/qjas.2021.129187.1000
[10] Ibrahim, H, I, Mahmoud. (2010). Plant samples collected and analyzed. First edition,. Al-Fajr Publishing House. and distribution. The Egyptian Arabic Republic.
[11] AL-Sahha, F. H. (1989b). Applied plant nutrition. Baghdad University. Ministry of Higher Education. and scientific research. Iraq.
[12] Jackson M.L., (1958). Soil Chemical analysis. Prentice Hall, Inc. Englewood Cliff, N.J. USA, P.225-276.
[13] Olsen S.R. and Sommers L.E., 1982. Phosphorus in A.L Page, (Ed). Methods of soil analysis. Part2. Chemical and Microbiological properties 2nd edition. Amer. Soc. of Agron. Inc. Soil Sci. Soc. Amer. Inc. Madison, Wis. U.S.A.
[14] AL-Mohammadi, S, M and Fadel, M.M. (2012). Statistics and Experiment Design. Dar. Osama for Publishing and Distribution. Ajman. Jordan.
[15] Al-Hadithi, M. O. (2015). Effect of different fertilization sources and Brassinosteroid (BR) growth regulator on growth and yield of apricot trees Prunus armeniacaL. PhD thesis, Department of Horticulture and Landscape Engineering, College of Agriculture, University of Baghdad.
[16] Mohammed, M.A., Salman, S.R., Abdulridha, W.M., (2020), Structural, optical, electrical and gas sensor properties of zro2 thin films prepared by sol-gel technique, NeuroQuantology, 18(3), pp. 22–27.
[17] Mussing C.,(2005) Brassinosteroid-promoted growth,Plant Biol.7:110-117 nutrient uptake in plants. Int. J. Curr Res. Aca. Rev. 2(12):142-154.
[18] Abu Zaid, S. N. (2000). Plant hormones and agricultural applications. Arabic House book. Publishing and Distribution. The Egyptian Arabic Republic.
[19] Nassar, Z. S. and Jamal, A., (2012). Effect of spraying PRO.SOL nutrient solution and licorice extract on some vegetative and flowering growth characteristics of Pelargonium zonale L. Kufa Journal of Agricultural Sciences. Volume (4) Issue (1) 53-43. Al-Munisi, Faisal Abdulaziz. 1975. Citrus, the scientific basis for its cultivation. First Edition - Dar. New Publications - Alexandria.