



Response of orange Saplings to spraying with brassinolide and adding organic fertilizer in some of vegetative and root growth characteristics

Israa Kareem Abdulhusein Alkanani, Harith Mahmood Azeez Al-Tamimi *

Horticulture and landscape Department, Agriculture College, University of Kerbala, Karbala, Iraq

*Corresponding author e-mail: harith.mhmod@uokerbala.edu.iq

<https://doi.org/10.59658/jkas.v11i3.2356>

Received: May 25, 2024	Abstract The experiment was carried out at the Horticulture station in the Al-Hindiya district / Karbala governorate during the 2023 growing season, using a Randomized Complete Block Design (RCBD) with three replications. The study aimed to investigate the response of two orange Cultivars (Blood and Navel) to spraying with the growth regulator brassinolide at three concentrations (0, 0.3 and 0.6 mg L ⁻¹) and the addition of organic fertilizer (Humzinc) to the soil at three concentrations (0, 0.5 and 1 g L ⁻¹). The results showed the superiority of the Blood Cultivar over the Navel Cultivar in stem length, stem diameter, Leaves number, root length, and root volume, while the Navel Cultivar excelled in leaf area. Treatment with brassinolide at a concentration of 0.6 mg L ⁻¹ or the addition of organic fertilizer at a concentration of 1 g L ⁻¹ resulted in the highest significant increase in stem length, stem diameter, Leaves number, leaf area, root length, and root volume. Regarding the interactions between study factors, the binary and ternary interactions had a significant effect on improving vegetative and root growth Traits. The ternary interaction treatment (Blood Cultivar + brassinolide 0.6 mg L ⁻¹ + 1 g L ⁻¹) outperformed by giving the highest average increase in stem length (35.98 cm), stem diameter (7.993 mm), average Leaves number (117.66 leaves seedling-1), root length (120.67 cm), and root volume (52.33 cm ³), while the treatment (Navel Cultivar + brassinolide 0.6 mg L ⁻¹ + 1 g L ⁻¹) significantly excelled in leaf area (38.57 cm ²).
Accepted: July 29, 2024	
Published: Sept. 15, 2024	
Keywords: Orange Saplings, brassinolide, organic fertilizer	

Introduction

Citrus are evergreen fruit trees belonging to the Rutaceae family, which includes many genera, most notably the commercially important Citrus genus, which encompasses the core groups of citrus fruits [1].

The cultivation of citrus trees is widespread in tropical and subtropical regions worldwide between 40° north and south of the equator [2]. Global citrus production amounts to 158,491,166 tons annually, cultivated over approximately 10,072,197 hectares. China leads in citrus production, followed by Brazil, India, Mexico, the United States, and Spain. Egypt ranks as the top citrus producer in the Arab world and seventh



globally [3]. The number of fruit-bearing citrus trees in Iraq is estimated at around 7,768,290 trees, producing approximately 1,761,17 tons, with Salah al-Din Governorate leading in production, followed by Baghdad and then Diyala Governorate [4].

The slow growth of orange sapling in the initial years post-budding, along with some failing to grow, coupled with their extended stay in the nursery for over a year, increases production and management costs [5]. This has prompted numerous researchers to seek suitable solutions to accelerate and enhance seedling growth by using various plant growth regulators and nutrient solutions for their significant role in plant growth. Among the crucial plant growth regulators is Brassinolide, which plays multiple roles in regulating various vital activities within plant cells [6]. Brassinolide is a plant hormone derived from the raw fatty extract of *Brassica napus* pollen grains, significantly contributing to plant growth and development, with effects synergism to auxins, gibberellins, and cytokinins, such as cell elongation, division, vascular differentiation, flowering, aging, stress tolerance, nucleic acid and protein synthesis [7].

Humic acid is a fundamental component for organic matter decomposition, playing a crucial role in enhancing the physical and chemical properties of soil. It serves as a source of nutrients for plants, particularly nitrogen [8]. The high acidity of humic acids improves soil efficiency, reduces nitrogen loss as ammonia gas, enhances ammonium representation efficiency, and improves carbon assimilation by increasing cell membrane permeability and phosphorus absorption [9]. Additionally, humic substances can carry mineral elements, especially microelements like iron, zinc, and manganese, making them more readily absorbable by plants [10]. Therefore, the research aims to investigate the brassinolide foliar applied and organic fertilizer addition in enhancing the vegetative and root growth characteristics and producing budded orange saplings suitable for transportation to the permanent location with minimal cost, time, and effort.

Materials and Methods

Experiment Location: The experiment was conducted at the Horticulture station in the Al-Hindiya district / Karbala governorate for the 2023 growing season to determine the effect of the growth regulator brassinolide and organic fertilizer on improving the vegetative and root growth characteristics of orange saplings of the Blood and Navel cultivars.

Research Preparation: A total of 270 orange Saplings, budded onto sour orange rootstock, were selected for uniform growth at six months old, planted in 1.25 kg polyethylene bags. On 20 February 2023, the sapling were transplanted into 26 cm diameter plastic pots filled with a mixed soil and peat moss blend in a 1:3 ratio, with a total soil weight of 10 kg.

Statistical Design and Study Factors: The research was conducted following a factorial experiment design (2*3*3) using a complete randomized design with three

replications, totaling 54 experimental units with 5 sapling per treatment The study factors were as follows:

Factor 1:

Represented by two orange Cultivars (Blood and Navel).

Factor 2:

The standard brassinolide solution was prepared by dissolving 1 g of the growth regulator in 1 litre of distilled water, then concentrations of (0, 0.3, and 0.6) mg L⁻¹ were prepared according to the dilution law.

$$\text{The volume} = \frac{\text{Required solution volume} \times \text{Required Solution Concentration}}{\text{Standard Solution Concentration}}$$

The sapling was sprayed in the early morning with the above concentrations on three dates: 15/3, 15/4, and 15/5/2023

Factor 3:

Addition of organic fertilizer humzinc containing the percentages shown in the table below at three concentrations (0, 0.5, and 1) g L⁻¹, applied in six installments from 15/3/2023 until 15/8/2023.

Table (1): Components of organic fertilizer humzinc (%)

chelated manganese	chelated zinc	chelated iron	Humic acid
1.5	2.5	3.5	40

Studied characteristics: Measurements were taken on 15/10/2023 as follows:

1- Main stem length increment (cm) 2- Main stem diameter increment (mm) 3- Average number of leaves (Leaf sapling⁻¹) 4- Average leaf area (cm²) As mentioned [11].

Leaf area = 0.66 * length * width

5- Average root length (cm) 6- Average root volume (cm³) estimated using the water displacement method as mentioned [12].

Results and Discussion

Main stem length increment (cm):

The results displayed in Table (2) indicate the superiority of the blood Cultivar by giving the highest average increase in the length of the main stem, reaching (21.60 cm), while the Navel Cultivar recorded the lowest average increase at (19.02 cm). Furthermore, the spraying treatment with the growth regulator brassinolide at a concentration of (0.6 mg L⁻¹) outperformed by giving the highest average increase in the length of the main stem, which was (28.21 cm) compared to the control treatment that gave the lowest average of (12.34 cm). As for the addition of humzinc fertilizer, the concentration of (1g L⁻¹) exceeded by giving the highest average increase in the length of the main stem at (23.76 cm) compared to the control treatment that gave the lowest average of (16.39 cm).

Regarding the interaction between the Cultivar and brassinolide, the results from the same table indicate significant differences in this trait. The treatment (blood +



brassinolide 0.6 mg L⁻¹) excelled by recording the highest average of (29.53 cm), which was not significantly different from the dual intervention treatment (Navel + brassinolide 0.6 mg L⁻¹) that reached 26.88 cm, while the treatment (Navel + brassinolide 0 mg L⁻¹) recorded the lowest average at (11.12 cm). As for the interaction between the Cultivar and humzinc fertilizer, the treatment (blood + humzinc 1g L⁻¹) excelled by recording the highest average increase in the length of the main stem at (25.59 cm) compared to the treatment (Navel + humzinc 0 g L⁻¹) which reached (15.70 cm). Additionally, the dual interaction treatment between brassinolide and humzinc had a significant effect on the average increase in the length of the main stem, as the treatment (brassinolide 0.6 mg L⁻¹ + humzinc 1 g L⁻¹) excelled by giving the highest average increase of (33.67 cm) compared to the control treatment for the dual intervention, which reached (9.38 cm).

The results from the same table indicate a significant difference in the interaction between study factors. The triple interaction treatment (blood + brassinolide 0.6 mg L⁻¹ + humzinc 1 g L⁻¹) outperformed by giving the highest mean increase of 35.98 cm compared to the treatment (Navel + brassinolide 0 mg L⁻¹ + humzinc 0 g L⁻¹) which yielded the lowest mean increase of 8.70 cm.

Cultivar X Brassinolide	Humzinc fertilizer g L ⁻¹			Brassinolide mg L ⁻¹	Orange Cultivar
	1	0.5	0		
13.56	16.33	14.27	10.07	0	Blood
21.72	24.47	23.07	17.63	0.3	
29.53	35.98	29.03	23.56	0.6	
11.12	13.23	11.43	8.70	0	Navel
19.04	21.20	18.70	17.23	0.3	
26.88	31.37	28.12	21.17	0.6	
2.673	4.630			LSD. 0.05	
Cultivar Average	Cultivar X Humzinc				
21.60	25.59	22.12	17.09	Blood	
19.02	21.93	19.42	15.70	Navel	
1.543	2.673			LSD. 0.05	
Brassinolide Average	Brassinolide X Humzinc				
12.34	14.78	12.85	9.38	0	
20.38	22.83	20.88	17.43	0.3	
28.21	33.67	28.58	22.37	0.6	
1.890	3.274			LSD. 0.05	
	23.76	20.77	16.39	Humzinc Average	
	1.890			LSD. 0.05	

Table (2): the effect of Cultivar, Brassinolide, adding Humzinc fertilizer, and their interaction on the Main stem length increment (cm)

Main stem diameter increment(mm)

The table (3) shows that there are no significant differences between the Blood and Navel Cultivars in the average increase in the diameter of the main stem. However, the spraying treatment with brassinolide at a concentration (0.6 mg L⁻¹) gave the highest



average increase in the diameter of the main stem (5.262 mm) compared to the control treatment, which gave the lowest average of (2.446mm). As for adding Humzinc fertilizer, the concentration (1 g L⁻¹) gave the highest average increase in the diameter of the main stem, which reached (4.759 mm) compared to the control treatment, which gave the lowest significant difference of (3.054 mm).

The results also showed that the binary interaction between the cultivar and brassinolide had a significant effect on the average increase in the diameter of the main stem, and the treatment (Blood + brassinolide 0.6 mg L⁻¹) recorded the highest average of (5.567 mm) compared to the control treatment (Blood + brassinolide 0 mg L⁻¹) which gave the lowest average of (2.810 mm). The results also showed that the binary interaction between the cultivar and Humzinc fertilizer had a significant effect on the average increase in the diameter of the main stem, as (Blood + Humzinc 1 g L⁻¹) gave the highest average increase of (5.009 mm) compared to the treatment (Blood + Humzinc 0 g L⁻¹) which gave the lowest value of (2.810 mm). As for the treatment of the binary interaction between brassinolide and Humzinc ,the treatment (brassinolide 0.6 mg L⁻¹ + Humzinc 1 g L⁻¹) gave the highest average increase in the diameter of the main stem, which reached (6.883 mm) compared to the treatment (brassinolide 0 mg L⁻¹ + Humzinc 0 g L⁻¹) which gave the lowest average increase in the diameter of the main stem of (1.858 mm).

The treatment of the triple interaction may have surpassed (Blood + brassinolide 0.6 mg L⁻¹ + Humzinc concentration 1 g L⁻¹) by giving the highest significant increase in the average increase in the diameter of the main stem, reaching (7.993 mm), compared to the treatment (Blood + brassinolide 0 mg l⁻¹ + Humzinc 0 g L⁻¹) which gave the lowest value of (1.630 mm).

Table (3): the effect of the Cultivar, spraying with brassinolide, adding Humzinc fertilizer, and their interaction on the Main stem diameter increment (mm).

Cultivar X Brassinolide	Humzinc fertilizer g L ⁻¹			Brassinolide mg L ⁻¹	Orange Cultivar
	1	0.5	0		
2.366	3.030	2.437	1.630	0	Blood
3.696	4.003	3.817	3.267	0.3	
5.567	7.993	5.173	3.533	0.6	
2.526	2.890	2.600	2.087	0	Navel
4.343	4.863	4.463	3.703	0.3	
4.957	5.773	4.993	4.103	0.6	
0.597	1.035			LSD. 0.05	
Cultivar Average	Cultivar X Humzinc				
3.942	5.009	3.809	2.810	Blood	
3.876	4.509	4.019	3.298	Navel	
N.S	0.597			LSD. 0.05	
Brassinolide Average	Brassinolide X Humzinc				
2.446	2.960	2.518	1.858	0	
4.019	4.433	4.140	3.485	0.3	
5.262	6.883	5.083	3.818	0.6	
0.422	0.732			LSD. 0.05	
	4.759	3.914	3.054	Humzinc Average	
	0.422			LSD. 0.05	

The average number of leaves (Leaf sapling⁻¹)

The table (4) shows a significant impact on the average increase in the number of leaves by the Cultivar, with the Blood Cultivar giving the highest average of (82.94 Leaf sapling⁻¹) compared to the Navel Cultivar, which gave the lowest value of (70.62 Leaf sapling⁻¹). Regarding the treatment with brassinolide, the concentration of (0.6 mg L⁻¹) outperformed by giving the highest average number of leaves at (91.46 Leaf sapling⁻¹) compared to the comparison treatment, which gave the lowest average of (62.35 Leaf sapling⁻¹). As for the treatment of adding humzinc, the treatment with a concentration of (1g L⁻¹) outperformed by recording the highest average number of leaves at (85.05 Leaf sapling⁻¹) compared to the control treatment, which gave the lowest value of (69.15 Leaf sapling⁻¹). The results of the same table also show that the interactions between the Cultivar and brassinolide have a significant impact on increasing the number of leaves. The treatment (Blood + brassinolide 0.6 mg L⁻¹) outperformed by giving a significant increase in the average number of leaves at (99.59 Leaf sapling⁻¹) compared to the treatment (Navel + brassinolide 0 mg L⁻¹) which recorded the lowest average of (57.52 Leaf sapling⁻¹). Similarly, the results of the interactions between the Cultivar and humzinc showed that the treatment (Blood + humzinc concentration 1g L⁻¹) significantly outperformed by giving the highest average number of leaves at (93.78 Leaf sapling⁻¹) compared to the treatment (Navel + humzinc 0g L⁻¹) which gave the lowest value of (64.97 Leaf sapling⁻¹). The treatment of the dual

interaction (brassinolide concentration 0.6 mg L^{-1} + Humzinc 1 g L^{-1}) showed the highest significant increase in the average number of leaves at ($103.79 \text{ Leaf sapling}^{-1}$) compared to the (brassinolide 0 mg L^{-1} + humzinc 0 g L^{-1}) which showed the lowest average of ($57.56 \text{ Leaf sapling}^{-1}$).

The results from the same table indicate that the triple interaction between the study factors has a significant effect on the average number of leaves. The treatment (Blood + brassinolide 0.6 mg L^{-1} + humzinc 1 g L^{-1}) outperformed by providing the highest average increase in the number of leaves ($117.66 \text{ Leaf sapling}^{-1}$), while the triple interaction treatment (Navel+ brassinolide 0 mg L^{-1} + humzinc concentration 0 g L^{-1}) recorded the lowest average at ($52.13 \text{ Leaf sapling}^{-1}$).

Table (4): Effect of Cultivar, spraying with the brassinolide, adding Humzinc fertilizer, and their interaction on the average number of leaves (Leaf sapling^{-1})

Cultivar X Brassinolide	Humizinc fertilizer g L^{-1}			Brassinolide mg L^{-1}	Orange Cultivar
	1	0.5	0		
67.18	70.67	67.88	62.99	0	Blood
82.03	93.00	79.66	73.44	0.3	
99.59	117.66	97.55	83.55	0.6	
57.52	64.11	56.31	52.13	0	Navel
71.03	74.93	72.93	65.22	0.3	
83.32	89.91	82.51	77.55	0.6	
5.798	10.042			LSD. 0.05	
Cultivar Average	Cultivar X Humzinc				
82.94	93.78	81.70	73.33	Blood	
70.62	76.32	70.58	64.97	Navel	
3.347	5.798			LSD. 0.05	
Brassinolide Average	Brassinolide X Humzinc				
62.35	67.39	62.10	57.56	0	
76.53	83.96	76.30	69.33	0.3	
91.46	103.79	90.03	80.55	0.6	
4.100	7.101			LSD. 0.05	
	85.05	76.14	69.15	Humzinc Average	
	4.100			LSD. 0.05	

Average leaf area (cm^2):

The results displayed in Table (5) show the superiority of the Navel Cultivar by giving the highest average leaf area of (32.64 cm^2) compared to the Blood Cultivar which gave the lowest average of (28.34 cm^2). As for the treatment with brassinolide, the concentration of (0.6 mg L^{-1}) recorded the highest average leaf area of (34.68 cm^2) compared to the control treatment (25.78 cm^2). When fertilized with humzinc at a concentration of 1 g L^{-1} , plants showed a significantly larger average leaf area of 32.96 cm^2 compared to the control treatment, which resulted in a smaller leaf area of 26.83 cm^2 .

The data in the table indicates that the Navel Cultivar combined with brassinolide had a notable impact on leaf area, particularly when treated with a brassinolide



concentration of 0.6 mg L⁻¹ outperformed by giving the highest average of (36.18 cm²) compared to the treatment (Blood Cultivar + brassinolide 0 mg L⁻¹) 22.21 cm². The findings revealed significant effects on the average leaf area due to the interactions between the different Cultivars and humzinc. Specifically, the treatment involving Navel Cultivar with a humzinc concentration of 1g L⁻¹ exhibited the highest average leaf area of 34.73 cm², whereas the treatment with Bloody Cultivar and humzinc concentration of 0g L⁻¹ showed the lowest average of 24.38 cm². Moreover, the combined treatment of (brassinolide concentration at 0.6 mg L⁻¹ + humzinc 1g L⁻¹) demonstrated a notable variance, yielding the highest average leaf area of 37.46 cm². In contrast, the treatment with (brassinolide 0 mg L⁻¹ + humzinc 0g L⁻¹) produced the lowest average leaf area of 23.73 cm².

When it comes to the triple interaction treatment, the table results indicate that the treatment (Navel + Brassnolide 0.6 mg L⁻¹ + Humzinc 1 g L⁻¹) outperformed by showing the highest average leaf area of 38.57 cm², whereas the treatment (Blood + Brassnolide 0 mg L⁻¹ + Humzinc 0 g L⁻¹) recorded the lowest average of 20.77 cm².

Table (5): Effect of Cultivar, spraying with the brassinolide, adding Humzinc fertilizer, and their interaction on the average leaf area (cm²)

Cultivar X Brassinolide	Humizinc fertilizer g L ⁻¹			Brassinolide mg L ⁻¹	Orange Cultivar
	1	0.5	0		
22.21	24.06	21.79	20.77	0	Blood
29.63	33.17	31.56	24.17	0.3	
33.18	36.34	34.98	28.21	0.6	
29.36	31.01	30.38	26.69	0	Navel
32.37	34.61	33.12	29.38	0.3	
36.18	38.57	38.19	31.79	0.6	
1.142	1.977			LSD. 0.05	
Cultivar Average	Humzinc X Cultivar				
28.34	31.19	29.44	24.38	Blood	
32.64	34.73	33.89	29.29	Navel	
0.659	1.142			LSD. 0.05	
Brassinolide Average	Brassinolide X Humzinc				
25.78	27.54	26.09	23.73	0	
31.00	33.89	32.34	26.78	0.3	
34.68	37.46	36.58	30.00	0.6	
0.807	1.398			LSD. 0.05	
	32.96	31.67	26.83	Humzinc Average	
	0.807			LSD. 0.05	

Average root length (cm):

Table (6) shows that various factors have a significant impact on the average root length. The Blood Cultivar excelled with the highest average root length of (73.26 cm),



surpassing the Navel Cultivar, which had the lowest average of (68.37 cm). The use of brassinolide at a concentration of 0.6 mg L⁻¹ resulted in the highest average root length of (93.56 cm), whereas the control treatment had the lowest average of (43.56 cm). Furthermore, the application of Humzinc at a concentration of 1 g L⁻¹ notably increased the average root length to (83.72 cm) compared to the control's (56.83 cm).

The results from the same table indicate a significant interaction between the type of Cultivar and brassinolid concerning the average root length. The treatment of (Blood + brassinolid 0.6 mg L⁻¹) performed the best, with the highest average of (98.11 cm), surpassing the Navel + brassinolid 0 mg L⁻¹ treatment, which had the lowest average root length of (42.89 cm). Similarly, the combined treatment of (Blood + humzinc 1 g L⁻¹) showed a significant increase in average root length of (85.22 cm) compared to the (Navel + humzinc 0 g L⁻¹) treatment, which had the lowest average of (53.67 cm). Additionally, the combined treatment of (brassinolid 0.6 mg L⁻¹ + humzinc 1 g L⁻¹) exhibited the most significant increase in average root length of (115.33 cm), in contrast to the treatment of (brassinolid 0 mg L⁻¹ + humzinc 0 g L⁻¹), which had the lowest value of (31.50 cm).

Moreover, the findings showed that the triple interaction treatments significantly influenced root length. The combination of (Blood+ brassinolide 0.6 mg L⁻¹ + humzinc 1 g L⁻¹) achieved the highest average root length of (120.67 cm), whereas the combination of (Navel+ brassinolide 0 mg L⁻¹ + humzinc 0 g L⁻¹) had the lowest average of (30.33 cm).

Table (6): Effect of the Cultivar, spraying with the brassinolide, adding Humzinc fertilizer, and their interaction on the average root length (cm).

Cultivar X Brassinolide	Humizinc fertilizer g L ⁻¹			Brassinolide mg L ⁻¹	Orange Cultivar
	1	0.5	0		
44.22	52.67	47.33	32.67	0	Blood
77.44	82.33	80.00	70.00	0.3	
98.11	120.67	96.33	77.33	0.6	
42.89	60.67	37.67	30.33	0	Navel
73.22	76.00	80.00	63.67	0.3	
89.00	110.00	90.00	67.00	0.6	
4.870	8.435			LSD. 0.05	
Cultivar Average	Cultivar X Humzinc				
73.26	85.22	74.56	60.00	Blood	
68.37	82.22	69.22	53.67	Navel	
2.812	4.870			LSD. 0.05	
Brassinolide Average	Brassinolide X Humzinc				
43.56	56.67	42.50	31.50	0	
75.33	79.17	80.00	66.83	0.3	
93.56	115.33	93.17	72.17	0.6	
3.444	5.965			LSD. 0.05	
	83.72	71.89	56.83	Humzinc Average	
	3.444			LSD. 0.05	

Average root volume (cm³):

The table (7) shows a significant impact of the Cultivar on the average root volume. The Blood Cultivar had the highest average volume at (37.22 cm³) compared to the Navel Cultivar, which had the lowest average at (35.37 cm³). The treatment with brassinolide at a concentration of 0.6 mg L⁻¹ resulted in the highest average root volume of (44.17 cm³) compared to the control treatment, which had the lowest average at (26.11 cm³). Furthermore, the addition of Humzinc fertilizer at a concentration of 1 g L⁻¹ recorded the highest average root volume of (40.67 cm³) compared to the control treatment, which had the lowest average at (30.72 cm³).

The results of the same table showed that binary interactions between the Cultivar and brassinolide had a significant effect on the average root volume. The combination (Blood + brassinolide 0.6 mg L⁻¹) had the highest average volume (45.22 cm³) compared to the treatment (Navel + brassinolide 0 mg L⁻¹) which had the lowest average root volume (25.00 cm³). The results indicated that binary interactions between the Cultivar and Humzinc had a significant effect on the average root volume. The combination (Blood + Humzinc 1 g L⁻¹) resulted in the highest average volume (43.00 cm³) compared to the treatment (Blood + Humzinc 0 g L⁻¹) which had the lowest average volume (30.33 cm³). As for the binary interaction treatment between brassinolide and hum zinc, the combination (brassinolide concentration 0.6 mg L⁻¹ + Humzinc 1 g L⁻¹) showed a significant increase in the average root volume (49.50 cm³) compared to the treatment (brassinolide concentration 0 mg L⁻¹ + Humzinc 0 g L⁻¹) which had the lowest average (22.83 cm³).

When it comes to the triple interaction treatment, the table results indicate that the treatment (Blood + brassinolide 0.6 mg L⁻¹ + Humzinc 1 g L⁻¹) showed superior performance by yielding the highest average root volume of (52.33 cm³). In contrast, the treatment (Navel + brassinolide 0 mg L⁻¹ + Humzinc 0 g L⁻¹) recorded the lowest average of (22.33 cm³).

Table (7) : Effect of Cultivar, spraying with the brassinolide, adding Humzinc acid, and their interaction on the average root volume (cm³)

Cultivar X Brassinolide	Humzinc fertilizer g L ⁻¹			Brassinolide mg L ⁻¹	Orange Cultivar
	1	0.5	0		
27.22	32.33	26.00	23.33	0	Blood
39.22	44.33	40.67	32.67	0.3	
45.22	52.33	48.33	35.00	0.6	
25.00	27.67	25.00	22.33	0	Navel
38.00	40.67	40.00	33.33	0.3	
43.11	46.67	45.00	37.67	0.6	
2.785	4.823			LSD. 0.05	
Cultivar Average	Cultivar X Humzinc				
37.22	43.00	38.33	30.33	Blood	
35.37	38.33	36.67	31.11	Navel	



1.608	2.785			LSD. 0.05
Brassinolide Average	Brassinolide X Humzinc			
26.11	30.00	25.50	22.83	0
38.61	42.50	40.33	33.00	0.3
44.17	49.50	46.67	36.33	0.6
1.969	3.411			LSD. 0.05
	40.67	37.50	30.72	Humzinc Average
	1.969			LSD. 0.05

The data presented highlights the superiority of the Blood Cultivar over the Navel Cultivar in most vegetative and root traits in tables (2, 3, 5, and 6), while the Navel Cultivar excelling in leaf area compared to the blood Cultivar in table (4). This difference is likely influenced by genetic variations, growth characteristics of both Cultivars, and their response to environmental conditions affecting plant physiology, photosynthesis efficiency, carbohydrate synthesis rate, and essential hormones for cell division and elongation, which collectively promote overall growth [5].

The enhancement of vegetative and root growth traits following treatment with the growth regulator brassinolide can be attributed to the significant role of brassinolide in regulating cell elongation. This regulation occurs through processes such as coordinated changes in cell wall properties, gene expression, and biochemical processes [13]. In most dicot and monocot plants, the primary cell wall comprises fine cellulose fibers, and brassinosteroids play a role in relaxing the cell wall by activating wall-bound enzymes like Expansion and Cellulose Synthase, thereby increasing cell flexibility [7]. Perhaps the reason lies in the involvement of brassinosteroids in encoding cyclins, especially Cyc-D3 and B-Type cyclins, leading to increased cell wall flexibility, cell division, and consequently enhanced plant cell growth [14], which positively reflects in the increase of vegetative and root growth indicators. The results align with [15,16] when spraying orange and fig sapling with the growth regulator brassinolide.

The enhanced vegetative and root growth observed in the data tables (2,3,4,5,6) following the application of Humzinc fertilizer can be credited to the fertilizer's components, especially its humic acid content. Humic acid improves membrane permeability, aiding nutrient transfer that activates serine with the indole ring to produce tryptophan, a precursor of the auxin hormone (IAA), This process promotes increased plant cell division or elongation [17,18]. Additionally, the presence of essential trace elements (Zn, Mn, Fe) in the fertilizer contributes to this effect by supporting key physiological functions in photosynthesis and plant hormone synthesis, thereby stimulating cell division and elongation [19]. These combined mechanisms positively impact and enhance the characteristics of vegetative and root growth. The findings align with [20,21] regarding the role of organic fertilizer (humic acid) in enhancing the growth of Egyptian lemon and olive trees successively.



References

- 1) Ibrahim, A. M. (2015). *Fruits and vegetables and human health*. Ma'aref Establishment. Alexandria, Arab Republic of Egypt.
- 2) Ismail, M., & Zhang, J. (2004). Post-harvest citrus diseases and their control. *Outlooks on Pest Management*, 15(1), 29.
- 3) Food and Agriculture Organization. (2021). *FAOSTAT agricultural statistics database*. <http://www.fao.org>
- 4) Central Statistical Organization and Information Technology. (2020). *Citrus trees production report*. Ministry of Planning and Development Cooperation, Baghdad, Iraq.
- 5) Al-Deiri, N. (2002). *Fruit trees are evergreen*. Publications of the University of Aleppo, Directorate of University Books and Publications.
- 6) Chai, Y., Qing, Z., & Lin, T. (2013). Brassinosteroid is involved in strawberry fruit ripening. *Plant Growth Regulation*, 69, 63–69.
- 7) Al-Khafaji, M. A. (2014). *Plant growth regulators: Application and utilization in horticulture*. Bookstore for Printing Publishing and Translating, University of Baghdad, Iraq.
- 8) Abd El-Razek, E., Haggag, L. F., El-Hady, E. S., & Shahin, M. F. M. (2020). Effect of soil application of humic acid and bio-humic on yield and fruit quality of “Kalamata” olive trees. *Bulletin of the National Research Centre*, 44(1), 1-8.
- 9) Trevisan, S., Francioso, O., Quaggiotti, S., & Nardi, S. (2010). Humic substances biological activity at the plant-soil interface: From environmental aspects to molecular factors. *Plant Signaling & Behavior*, 5(6), 635-643.
- 10) Abu Aiyana, R. A., Alfada, S. A., & Al-Redhiman, K. N. (2013). *Organic palm agriculture*. Saudi Arabia.
- 11) Chou, G. J. (1966). A new method of measuring the leaf area of citrus. *Acta Horticulturae Scientiarum*, 5, 7-20.
- 12) Al-Tamimi, H., Lateef, S., & Mahmood, O. (2023). Effect of foliar spraying with Nano-NPK fertilizer on some growth characteristics and chemical content of some citrus rootstocks. *Revis Bionatura*, 8(3), 116.
- 13) Shahbaz, M., & Ashraf, M. (2007). Influence of exogenous application of brassinosteroid on growth and mineral nutrients of wheat (*Triticum aestivum* L.) under saline conditions. *Pakistan Journal of Botany*, 39, 513-522.
- 14) Hayat, S., & Ahmed, A. (2011). *Brassinosteroids: A class of plant hormone*. Springer.
- 15) Zulkarnaini, Z. M., Zaharah, S. S., Mohamed, M. T. M., & Jaafar, H. Z. (2019). Effect of brassinolide application on growth and physiological changes in two cultivars of fig (*Ficus carica* L.). *Pertanika Journal of Tropical Agricultural Science*, 42(1), 95-105.
- 16) Al-Ahbaby, A. J. A., & Al-Ani, T. K. G. (2021). The effect of foliar spraying with some growth stimulants on improving vegetative growth and mineral content of



- seedlings of navel orange and blood orange. In *IOP Conference Series: Earth and Environmental Science* (Vol. 923, No. 1, p. 012005). IOP Publishing.
- 17) Ali, A., Fleih, S., Idan, R., & Aziz, H. (2017). Response of olive seedlings to treatment with licorice and yeast extract. *Journal of Kerbala for Agricultural Sciences*, 4(4), 56-68.
- 18) Al-Tememe, Z. M. A., Al-Tamimi, H. M. A., Al-Asedy, A. A. A., & Al-Amirry, A. T. (2018). Effect of humic acid and seaweed extract and foliar application on some fruit growth characteristics of olive (*Olea europaea* L.) cv. Bashiq. *Journal of Kerbala for Agricultural Sciences*, 5(5), 276-288.
- 19) Jones Jr, J. B. (2012). *Plant nutrition and soil fertility manual* (2nd ed.). CRC Press.
- 20) Ennab, H. A. (2018). Effect of humic acid on growth and productivity of Egyptian lime trees (*Citrus aurantifolia* Swingle) under salt stress conditions. *Journal of Agricultural Research Kafr El-Sheikh University*, 42(4), 494-505.
- 21) Al-Qady, R. A., Hussein, S. A., & Albayati, M. M. (2018). Effect of organic fertilization with humic acid and the growth regulator naphthalene acetic acid (NAA) on some growth characteristics of olive (*Olea europaea* L.) variety Bashyki. *Tikrit Journal for Agricultural Sciences*, 18(1), 55-63.