



Research Article

The Effect of the Brassinolide Growth Regulator and Organic Fertilizer on The Nutritional and Mineral Elements of Orange Seedlings

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Abstract | The fruit tree planting business has continually experienced growth because of increasing demand for fresh, locally grown fruits. This experiment was carried out at the Horticulture Station located in the Al-Hindiya district of Kerbala, Iraq, during the 2023 growing season. A completely randomized design with three replications was employed. This study intended to assess the response of two orange varieties, Blood and Navel, when subjected to foliar application of the growth regulator Brassinolide at three levels (0, 0.3, and 0.6 mg/L) and the addition of HumZinc to the soil at three different levels (0, 0.5, and 1 g/L) with the purpose of improving the nutritional and mineral seedlings' contents. The results showed that the Blood variety had all nutritional contents higher than the Navel variety. The treatment with Brassinolide at 0.6 mg/L or the application of HumZinc at 1 g/L was the most influential with regard to the increases in leaf content of carbohydrates, proteins, and nitrogen (N). The interactions among the factors studied were also significant as contributors to better nutritional and chemical properties of the orange seedlings. The highest yield was recorded when the Blood variety was treated with 0.6 mg/L Brassinolide and 1 g/L HumZinc, as it showed the highest amount of carbohydrates 19.10%, proteins 16.67%, and nitrogen 2.67 % in leaves, respectively. In conclusion, the application of either foliar Brassinolide or soil-applied HumZinc or in combination considerably enhanced the nutritional value both Blood and Navel orange seedlings.

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Introduction

Orange trees belong to the Rutaceae family and the Citrus genus, which comprises various species and varieties that adapted to different environmental conditions (Ibrahim, 2015). The fruit tree planting business has grown drastically as people

increasingly demand fresh, locally produced fruits. Orange production constitutes around two-thirds of the global citrus output, which stands at around 158.5 million tonnes per year (FAO, 2020). The orange is in high demand due to its sweet taste, bitterness-free, and versatility in terms of usage as fresh fruit or juice. Besides, oranges are highly processed into canned

and frozen fruits that consume an enormous part of the world's production and are still increasing in the markets. In Iraq, there are approximately 6.38 million fruit-bearing orange trees, producing around 142,717 tons per year, while Salah al-Din Governorate is the largest one, followed by Baghdad and Diyala Governorates (Central Statistical Organization, 2020). This is mainly because orange trees in Iraq produce relatively low amounts of yield. Farmers depend on local oranges, which have lower yields than international ones. To increase production, it is significant to pay attention to high-yielding international varieties, such as the Blood and Navel type, which have already proved successful in Iraq (Al-Ahbaby and Al-Ani, 2021). Orange seedlings planted outside in the field grow very slowly in the first years after grafting and even some seedlings fail to grow. Moreover, such seedlings stay for more than a year in the nursery. This increases the cost of production and also limits controlling the growth process (Al-Deri, 2002). Thus, it has been found that plant growth regulators combined with organic fertilizers provide an effective means to better grow plants and regulate their development. Brassinolide is a growth regulator that has been the focus of interest as it improves cellular elongation, division, and differentiation of conducting vessels besides lateral shoot growth, synthesis of carbohydrate in leaves, and delays senescence (Tang *et al.*, 2016). Organic fertilizers like HumZinc also promote plant growth by enhancing soil properties such as aeration, structure, water retention capacity. Cation exchange capacity (CEC), and microbial activity. Directly, organic fertilizers stimulate plant growth similarly to plant hormones. While the exact mechanisms remain unclear, one hypothesis suggests that organic acids enhance cell wall permeability, improving nutrient uptake, particularly in root cells, and promoting respiration, photosynthesis, phosphorus absorption, and cell expansion (Nardi *et al.*, 2009; Canellas and Olivares, 2014; Zafar *et al.*, 2024). The present study is designed with the hypothesis that the foliar application of plant growth regulator Brassinolide and the addition of organic fertilizer HumZinc to the soil will significantly improve the nutritional and mineral content of orange seedlings. The objective of this research is to determine the most effective combination of Brassinolide and HumZinc that will eventually produce healthy seedlings to be transplanted into their permanent location.

Materials and Methods

This experiment was conducted at the Horticulture Station in Al-Hindiya district, Kerbala, Iraq, during the growing season 2023. The experiment had a complete randomized design with three replications. The objective of the study was to evaluate two orange varieties-Blood and Navel. Responses to foliar application of a growth regulator, Brassinolide at three concentrations (0, 0.3, and 0.6 mg/L), and the addition of HumZinc to the soil at three concentrations (0, 0.5, and 1 g/L) to enrich seedlings' nutritional and mineral qualities.

Experiment location

The experiment was carried out at the Horticulture Station within the Al-Hindiya district, Karbala Governorate, Iraq, at coordinates 32° 32' 26.89" N, 44° 10' 28.62" E, during the year of the 2023 growing season. The purpose was to examine the effects of the foliar spray of the growth regulator Brassinolide and the incorporation of organic fertilizer, HumZinc, on the nutritional and chemical quality of two orange varieties of seedlings, Blood and Navel.

Research preparation

The 270 orange seedlings, grafted onto sour orange rootstock and six months in age, were used in this investigation for uniform growth. The seedlings were grown in 1.25 kg polyethylene bags. On 20th February 2023, the seedlings were transferred to 26 cm diameter plastic pots. A mixture of soil and peat moss with a ratio of 1:3 was put into the pot, and the total weight of the soil used for each pot was 10 kg.

Factors under study

There were three main factors for study.

Variety of orange seedling: There were two varieties utilized, which were Blood and Navel.

Plant growth regulator brassinolide: The seedlings were administered with Brassinolide at three levels of concentration: 0, 0.3, and 0.6 mg/L. The seedlings were sprayed with Brassinolide on 15 March, 15 April, and 15 May, 2023 early morning.

HumZinc organic fertilizer: HumZinc was applied to the soil in three concentrations (0, 0.5, and 1 g/L). Six applications of fertilizer were spread between 15 March to 15 August, 2023.

Studied attributes

On October 25, 2023, following measurements were taken on several characteristics of the seedlings.

Total chlorophyll contents

Chlorophyll meter (SPAD-502, Minolta, Japan) was used to obtain readings estimating leaf chlorophyll concentration (SPAD value). Five plants per treatment were selected randomly and SPAD values were recorded from the fully matured leaves counted from the top of the plants, the youngest fully expanded leaf. The observations were recorded from each pot.

Total soluble carbohydrates (%)

Determined according to Joslyn (1970).

Total protein content (%)

Protein content was estimated using the formula proposed by Al-Tamimi *et al.* (2023).

Protein in leaves = Percentage of Nitrogen (N) in the leaves * 6.25.

Nitrogen concentration (%)

Nitrogen content was measured using a Micro Kjeldahl apparatus according to the method proposed by Novozamsky *et al.* (1974). Phosphorus content was quantified using a spectrophotometer based on the method described by John (1970). Potassium concentration was analyzed by Flame Photometer according to the method proposed by Hesse and Hesse (1971).

Data analysis

The analysis was done with factorial experiment design (2×3×3) which was conducted using completely randomized design (CRD) based on three replications. Thus, it formed 54 experimental units with five seedlings in each treatment.

Results and Discussion

Results

The results presented in Table 1 exhibited that variety has a significant effect on the chlorophyll content in the leaves. Blood variety has the highest chlorophyll content at 70.86%, while Navel variety has lower chlorophyll content at 67.37%. When the plant growth regulator Brassinolide was applied, the concentration of 0.6 mg/L resulted in the highest chlorophyll content at 78.23% in comparison to the control group

of 60.89%. A significant effect was also observed in interaction between the variety and Brassinolide, at 0.6 mg/L, where the blood variety had the highest chlorophyll content at 81.24%, and the control for the Blood variety with no Brassinolide showed the lowest at 62.49%. The interaction between the variety and HumZinc also produced significant results, with the Blood variety and HumZinc at 1 g/L giving the highest chlorophyll content of 76.73%, compared to the Navel variety with no HumZinc, which recorded the lowest at 61.24%.

The highest percentage of 85.97% was obtained when Brassinolide at 0.6 mg/L combined with HumZinc at 1 g/L, while the control had the lowest at 56.17%. With the triple interaction, the cumulative chlorophyll content was at 88.04% with the combination of Blood variety, Brassinolide at 0.6 mg/L, and HumZinc at 1 g/L, while the lowest chlorophyll content at 58.58% was recorded from the triple interaction treatment of the Navel variety with no treatments.

Table 1: Effect of Brassinolide spraying and HumZinc, and their interaction on variety, in the total chlorophyll content of the leaves (SPAD).

Orange Variety	HumZinc fertilizer g L ⁻¹				Variety *
	Brassinolide mg L ⁻¹	0	0.5	1	Brassinolide
Blood	0	58.58	63.33	65.57	62.49
	0.3	61.83	68.10	76.57	68.83
	0.6	68.99	86.69	88.04	81.24
Navel	0	53.76	61.59	62.51	59.28
	0.3	63.73	67.93	71.14	67.60
	0.6	66.23	75.54	83.89	75.22
LSD. 0.05		5.360		3.094	
Variety * HumZinc					Variety Average
Blood		63.13	72.71	76.73	70.86
Navel		61.24	68.36	72.51	67.37
LSD. 0.05		3.094		1.787	
Brassinolide * HumZinc					Brassinolide Average
0		56.17	62.46	64.04	60.89
0.3		62.78	68.02	73.86	68.22
0.6		67.61	81.12	85.97	78.23
LSD. 0.05		3.790		2.188	
HumZinc Average		62.19	70.53	74.62	
		2.188			

Percentage of carbohydrates in leaves (%)

The results presented in Table 2 exhibited that variety has a significant effect on the carbohydrate content in the leaves. Blood variety has the highest carbohydrate content at 14.43%, while Navel variety has lower carbohydrate content at 12.35%. When the plant growth regulator Brassinolide was applied, the concentration of 0.6 mg/L resulted in the highest carbohydrate percentage at 15.45% in comparison to the control group of 11.37%. A significant effect was also observed in interaction between the variety and

Brassinolide, at 0.6 mg/L, where the blood variety had the highest carbohydrate content at 16.71%, and the control for the blood variety with no Brassinolide showed the lowest at 12.19%. The interaction between the variety and HumZinc also produced significant results, with the blood variety and HumZinc at 1 g/L giving the highest carbohydrate percentage of 16.23%, compared to the Navel variety with no HumZinc, which recorded the lowest at 10.90%. The highest carbohydrate percentage of 17.42% was obtained when Brassinolide at 0.6 mg/L combined with HumZinc at 1 g/L, while the control had the lowest at 9.84%. With the triple interaction, the cumulative carbohydrate content was at 19.10% with the combination of Blood variety, Brassinolide at 0.6 mg/L, and HumZinc at 1 g/L, while the lowest carbohydrate percentage at 10.15% was recorded from the triple interaction treatment of the Navel variety with no treatments.

Table 2: *Effect of Brassinolide spraying and HumZinc, and their interaction on variety, in the percentage of carbohydrates in leaves.*

Orange Variety	HumZinc fertilizer g L ⁻¹			Variety * Brassinolide
	Brassinolide mg L ⁻¹	0	0.5	1
Blood	0	10.15	12.63	13.78
	0.3	12.13	15.19	15.80
	0.6	13.66	17.38	19.10
Navel	0	9.52	10.67	11.48
	0.3	11.16	11.89	13.83
	0.6	12.03	14.80	15.75
LSD. 0.05		1.820		1.050
		Variety * HumZinc		Variety Average
Blood		11.98	15.07	16.23
Navel		10.90	12.45	13.69
LSD. 0.05		1.050		0.607
		Brassinolide * HumZinc		Brassinolide Average
0		9.84	11.65	12.63
0.3		11.65	13.54	14.82
0.6		12.84	16.09	17.42
LSD. 0.05		1.287		0.743
HumZinc		11.44	13.76	14.96
Average			0.743	12.35

Leaf percentage protein (%)

The results displayed in Table 3 exhibited significant differences between the Blood and Navel varieties in terms of protein content in the leaves. The blood variety outperformed the Navel variety, with a protein percentage of 10.83% compared to 9.91% for Navel. The Brassinolide treatment at 0.6 mg/L resulted in the highest protein percentage at 12.72%, while the control treatment had the lowest protein percentage at 8.15%. When HumZinc was incorporated at 1 g/L, the highest protein percentage was seen to be 12.10%, as opposed to the control treatment with a lower protein percentage of 7.88%. The binary interaction between the blood variety and Brassinolide at 0.6 mg/L showed the highest increase in protein

percentage at 13.41%, while the Navel variety with no Brassinolide had the lowest protein percentage at 7.83%. The blood variety with HumZinc at 1 g/L also had the highest protein percent with 12.79%, while the Navel variety with no HumZinc had the lowest at 7.77%. The combination of Brassinolide at 0.6 mg/L and HumZinc at 1 g/L had the highest protein content at 15.65%, while the control treatment had the lowest with 7.25%. The triple interaction of Blood variety with Brassinolide at 0.6 mg/L and HumZinc at 1 g/L had the highest protein percent at 16.67%, while that of Navel variety that received no treatment was the lowest at 7.04%.

Table 3: *Effect of Brassinolide spraying and HumZinc, and their interaction on variety, in the percentage of protein in leaves.*

Orange Variety	HumZinc fertilizer g L ⁻¹			Variety * Brassinolide
	Brassinolide	0	0.5	1
Blood	0	7.458	8.708	9.250
	0.3	7.979	11.396	12.458
	0.6	8.500	15.063	16.667
Navel	0	7.042	7.979	8.479
	0.3	7.917	10.583	11.125
	0.6	8.354	13.084	14.625
LSD 0.05		0.9610		0.5549
		Variety * HumZinc		Variety Average
Blood		7.979	11.722	12.792
Navel		7.771	10.549	11.410
LSD 0.05		0.5549		0.5194
		Brassinolide * HumZinc		Brassinolide Average
0		7.250	8.344	8.865
0.3		7.948	10.990	11.792
0.6		8.427	14.073	15.646
LSD 0.05		0.6796		0.4579
HumZinc		7.875	11.136	12.101
Average			0.3923	10.37

Nitrogen concentration in leaves (%)

The results presented in Table 4, showed the effect of variety on nitrogen percentage concentration in the leaves is available. The blood variety had a higher nitrogen concentration at 1.73% as compared to the navel variety 1.59%. The treatment with Brassinolide at 0.6 mg/L gave the highest concentration of nitrogen at 2.03% as compared to the control at 1.30%. Interaction between the variety and Brassinolide had a significant effect on nitrogen concentration. The highest nitrogen content was shown by a combination of blood variety and Brassinolide at 0.6 mg/L, with a content of 2.15%, but the lowest was recorded in the Navel variety with no Brassinolide at 1.25%. The interaction between the variety and HumZinc at 1 g/L recorded the highest nitrogen concentration at 2.50%, compared to the navel variety with no HumZinc with lowest value at 1.24%. The highest nitrogen concentration was recorded in the combination of Brassinolide at 0.6 mg/L and HumZinc at 1 g/L, at 2.50% followed

by control treatment with no Brassinolide and no HumZinc, recording the lowest value at 1.16%. In the triple interaction, the treatment combining the blood variety, Brassinolide at 0.6 mg/L, and HumZinc at 1 g/L achieved the highest concentration of nitrogen, at 2.67%, while the combination of navel variety with no treatments recorded the lowest concentration of nitrogen, at 1.13%.

Table 4: *Effect of Brassinolide spraying and HumZinc, and their interaction on variety, in Nitrogen concentration in leaves (%).*

Orange Variety	Brassinolide mg L ⁻¹	HumZinc fertilizer g L ⁻¹			Variety * Brassinolide
		0	0.5	1	
Blood	0	1.193	1.393	1.480	1.356
	0.3	1.277	1.823	1.993	1.698
	0.6	1.360	2.410	2.667	2.146
Navel	0	1.127	1.277	1.357	1.253
	0.3	1.267	1.693	1.780	1.580
	0.6	1.337	2.093	2.340	1.923
LSD. 0.05		0.1538			0.0888
Variety * HumZinc					
Blood		1.277	1.876	2.047	1.733
Navel		1.243	1.688	1.826	1.586
LSD. 0.05		0.0888			0.0513
Brassinolide * HumZinc					
0		1.160	1.335	1.418	1.304
0.3		1.272	1.758	1.887	1.639
0.6		1.348	2.252	2.503	2.034
LSD. 0.05		0.1087			0.0628
HumZinc Average		1.260	1.782	1.936	1.659
		0.0628			0.5679

Phosphorous concentration in leaves (%)

The results presented in Table 5, showed the effect of variety on phosphorous percentage concentration in the leaves is available. The Blood variety had a higher phosphorous concentration at 0.15% as compared to the Navel variety 0.13%. The treatment with Brassinolide at 0.6 mg/L gave the highest concentration of phosphorous at 0.162% as compared to the control at 0.13%. Interaction between the variety and Brassinolide had a significant effect on phosphorous concentration. The highest phosphorous content was shown by a combination of blood variety and Brassinolide at 0.6 mg/L, with a content of 2.15%, but the lowest was recorded in the Navel variety with no Brassinolide at 1.25%. The interaction between the variety and HumZinc at 1 g/L recorded the highest phosphorous concentration at 0.18%, compared to the Navel variety with no HumZinc with lowest value at 0.11%. The highest phosphorous concentration was recorded in the combination of Brassinolide at 0.6 mg/L and HumZinc at 1 g/L, at 0.175% followed by control treatment with no Brassinolide and no HumZinc, recording the lowest value at 0.09%.

Table 5: *Effect of Brassinolide spraying and HumZinc, and their interaction on variety, in Phosphorous concentration in leaves (%).*

Orange Variety	Brassinolide mg L ⁻¹	HumZinc fertilizer g L ⁻¹			Variety * Brassinolide
		0	0.5	1	
Blood	0	0.1	0.15	0.17	0.14
	0.3	0.12	0.16	0.18	0.15
	0.6	0.14	0.18	0.19	0.17
Navel	0	0.08	0.12	0.14	0.11
	0.3	0.11	0.13	0.15	0.13
	0.6	0.13	0.17	0.16	0.15
LSD. 0.05		0.0720			0.050
Variety * HumZinc					
Blood		0.12	0.16	0.18	Variety Average 0.15
Navel		0.11	0.14	0.15	0.13
LSD. 0.05		1.050			0.607
Brassinolide * HumZinc					
0		0.09	0.135	0.155	0.13
0.3		0.115	0.145	0.165	0.142
0.6		0.135	0.175	0.175	0.162
LSD. 0.05		1.287			0.743
HumZinc Average		0.113	0.152 0.743	0.165	0.143

Table 6: *Effect of Brassinolide spraying and HumZinc, and their interaction on variety, in Potassium concentration in leaves (%).*

Orange Variety	HumZinc fertilizer g L ⁻¹			Variety * Brassinolide	
	Brassinolide mg L ⁻¹	0	0.5	1	
Blood	0	0.9	0.15	1.3	0.78
	0.3	1.1	0.16	1.7	0.98
	0.6	1.2	0.18	1.8	1.06
Navel	0	0.7	0.12	1.1	0.64
	0.3	0.8	0.13	1.4	0.77
	0.6	1.0	0.17	1.5	0.89
LSD. 0.05		1.820			1.050
		Variety * HumZinc			Variety Average
Blood		1.06	0.163	1.66	0.961
Navel		0.83	0.14	1.33	0.76
LSD. 0.05		1.050			0.607
		Brassinolide * HumZinc			Brassinolide Average
0		0.8	0.135	1.2	0.71
0.3		0.95	0.145	1.55	0.88
0.6		1.1	0.175	1.65	0.97
LSD. 0.05		1.287			0.743
HumZinc Average		0.95	13.76	1.47	0.85
		0.743			

Potassium concentration in leaves (%)

The results presented in Table 6, showed the effect of variety on potassium percentage concentration in the leaves is available. The blood variety had a higher potassium concentration at 0.96% as compared to the navel variety 0.76%. The treatment with Brassinolide at 0.6 mg/L gave the highest concentration of potassium at 0.97% as compared to the control at 0.71%. The highest nitrogen content was shown by a combination of blood variety and Brassinolide at 0.6 mg/L, with a content of 1.06%, but the lowest was recorded in the navel variety with no Brassinolide at 0.83%. The interaction between the variety and HumZinc at 1 g/L recorded the highest potassium concentration at 1.8%, compared to the navel variety with no HumZinc with lowest value at 1%. In the

triple interaction, the treatment combining the blood variety, Brassinolide at 1.06 mg/L, and HumZinc at 1 g/L achieved the highest concentration of potassium, at 1.8%, while the combination of Navel variety with no treatments recorded the lowest concentration of potassium, at 0.83%.

Dendrogram, heat map and principal component analysis

Dendrogram: The cluster analysis grouped the attributes (Figure 1). Pprotein and carbohydrate percentage in leaves were grouped in a single cluster based on similarities with application of foliar Brassinolide and Hum Zinc. The nitrogen, phosphorous and potassium were placed in another cluster and chlorophyll contents in 3rd cluster (Zafar *et al.*, 2015; Tahir *et al.*, 2022).

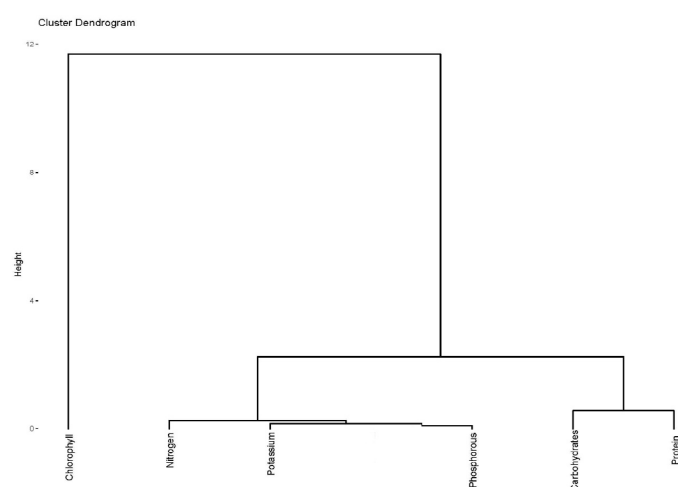


Figure 1: Dendrogram/Cluster analysis grouping of biochemical attributes by Brassinolide spraying and HumZinc, and their interaction on orange variety Blood and Navel.

Heat map

Histogram analysis was performed to illustrate the relationship amongst attributes with application of foliar Brassinolide and Hum Zinc. Remarkable distinctions were determined. The cyan color indicating the non-significant difference in treatments while other colors showed the significant difference in histogram study (Figure 2).

Histogram analysis indicating a considerable effective correlation of potassium, phosphorous, nitrogen, carbohydrates, proteins and total chlorophyll contents (Zafar *et al.*, 2019).

Heat map correlation histogram showing various attributes of orange variety Blood with foliar application of brassinolide 0, HumZinc 0 (1), brassinolide 0.3, HumZinc 0 (2), brassinolide 0.6,

HumZinc 0 (3), brassinolide 0, HumZinc 0.5 (4), brassinolide 0.3, HumZinc 0.5 (5), brassinolide 0.6, HumZinc 0.5 (6), brassinolide 0, HumZinc 1 (7), brassinolide 0.3, HumZinc 0.5 (8), brassinolide 0.6, HumZinc 1 (9), for variety Navel brassinolide 0, HumZinc 0 (10), brassinolide 0.3, HumZinc 0 (11), brassinolide 0.6, HumZinc 0 (12), brassinolide 0, HumZinc 0.5 (13), brassinolide 0.3, HumZinc 0.5 (14), brassinolide 0.6, HumZinc 0.5 (15), brassinolide 0, HumZinc 1 (16), brassinolide 0.3, HumZinc 1 (17), brassinolide 0.6, HumZinc 1 (18).

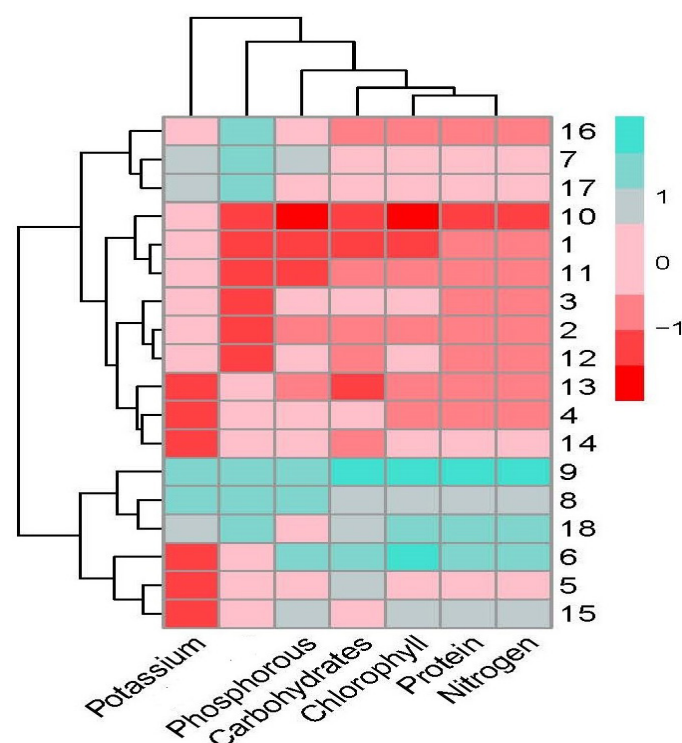


Figure 2: Heat map correlation histogram showing various biochemical attributes by Brassinolide spraying and HumZinc, and their interaction on orange variety Blood and Navel.

Principal component analysis

The plots of PCA showed the outcome of foliar treatments of fulvic organic fertilizer treatments and planting distances. A clear separation of various parameters was presented by Dim1 and Dim2 (Figure 3). In entire database, Dim 1 and Dim 2 illustrated the extreme influence and occupy more than 96.2% of whole databases, with Dim 1 shows 74.3% and Dim 2 shows 14.1%. A clear distinction of attributes with respect to brassinolide application and HumZinc was detected in PC analysis (Zafar *et al.*, 2019).

The data presented (Tables 1-6) indicated that blood variety has surpassed Navel in terms of all nutritional aspects. Such differences could be contributed to genetic differences, growth parameters

and their response to environmental factors. These features affect the physiological condition of the plant, chlorophyll contents, carbon metabolism, carbohydrates, hormone production, and mineral nutrient absorption, all these attributes contribute towards the overall growth in general (Al-Deiri, 2002). It increased the photosynthetic efficiency positively and showed higher carbohydrate accumulation in the blood variety.

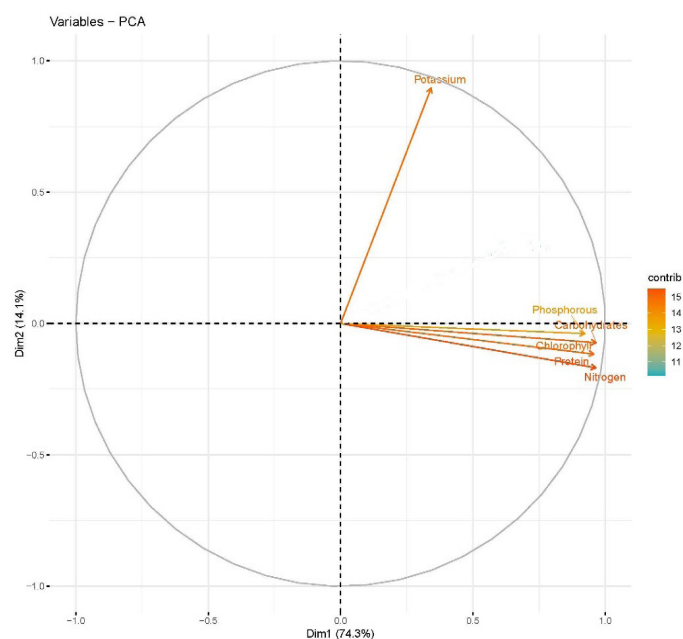


Figure 3: Principal component analysis exhibiting relationship between various biochemical attributes by Brassinolide spraying and HumZinc, and their interaction on orange variety Blood and Navel.

The increase in protein content in the leaves was probably due to the brassinolide effect that enhanced the nitrogen concentration. This may be due to the activation of brassinolide genes responsible for gibberellin synthesis in the root tips, stimulating growth in roots and improving mineral absorption. Additionally, as a steroid, brassinolide may bind to polyamines, helping to increase osmotic potential in plant cells and encouraging the roots to absorb more water and essential nutrients like nitrogen, phosphorus, and potassium (Khripach *et al.*, 2010; Ross and Quittenden, 2016). These findings are in line with those of Al-Ahbaby and Al-Ani (2021), as well as Lateef *et al.* (2023), who reported increases in carbohydrate, protein, and nitrogen content in orange saplings treated with brassinolide.

The rise in carbohydrate, protein, and nitrogen content upon the application of organic fertilizer HumZinc can be attributed to its constitutional composition, which include 40% humic acid and

micro-elements. Humic acid is known to increase cell membrane permeability, nutrient transfer, especially nitrogen, raising the potential ability of the plant to uptake and accumulate nutrients in the leaves (Farhan, 2008). Increased carbohydrate content can also be attributed to humic acid effect in stimulating chlorophyll production, ultimately increasing, photosynthesis efficiency. Furthermore, the micro-elements (Zn, Mn, Fe) present in HumZinc are essential to photosynthesis and to the activation of enzymes such as H-ATPase in the cell membrane. These enzymes enhance the uptake and translocation of mineral nutrients, especially nitrogen, within the vascular system of the plant, thereby producing more carbohydrate in the plant (Munson, 2018). These findings support those conducted by Tahira *et al.* (2013) and Ennab *et al.* (2023), which underscore the advantages of organic fertilizers such as humic acid.

Conclusions and Recommendations

In a nutshell, using foliar application of fertilizers as brassinolide or soil application of HumZinc at the specified concentrations as standalone treatments or in combinations, significantly improved the nutritional and chemical content of both Blood and Navel orange seedlings.

Novelty Statement

There is limited research on the combined effects of the plant growth regulator brassinolide and organic fertilizers in improving the nutritional and mineral content of orange seedlings, making them better suited for successful transplantation. This study introduces a new approach by exploring how brassinolide and organic fertilizers influence the growth, development, and mineral composition of orange seedlings, aiming to enhance their overall health and readiness for permanent planting.

Author's Contribution

Israa Kareem Abdulhussein Alkanani: Performed the immunological assays, wrote the first draft of the manuscripts.

Harith Mahmood Azeez Al-Tamimi: Designed the experimentation and analyzed the data.

Conflict of interest

The authors have declared no conflict of interest.

References

- Al-Ahbab, A.J.A. and T.K.G. Al-Ani. 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. IOP Conf. Ser. Earth Environ. Sci., 923(1): 012005. <https://doi.org/10.1088/1755-1315/923/1/012005>
- Al-Deiri, N., 2002. Fruit trees are evergreen. Publications of the University of Aleppo. Directorate of University Books and Publications, Syria.
- Al-Tamimi, H., S. Lateef and O. Mahmood. 2023. Effect of foliar spraying with Nano-NPK fertilizer in some growth characteristics and chemical content of some citrus rootstocks. Rev. Bionatura., 8(3): 116.
- Berk, Z., 2016. Citrus fruit processing. Academic press.
- Canellas, L.P. and F.L. Olivares. 2014. Physiological responses to humic substances as plant growth promoter. Chem. Biol. Technol. Agric., 1: 1-11. <https://doi.org/10.1186/2196-5641-1-3>
- Central Statistical Organization and Information Technology, 2020. Ministry of planning and development cooperation. Citrus Trees Production Report, Baghdad. Iraq.
- Ennab, H.A., A.H. Mohamed, H.M. El-Hoseiny, A.A. Omar, I.F. Hassan, M.S. Gaballah, S.E. Khalil, A.M. Mira, A.F. Abd El-Khalek and S.M. Alam-Eldein. 2023. Humic acid improves the resilience to salinity stress of drip-irrigated Mexican lime trees in saline clay soils. Agron., 13(7): 1680. <https://doi.org/10.3390/agronomy13071680>
- FAO, 2020. FAOSTAT database agricultural statistics database www.fao.org/publications.Rome.
- Farhan, H.N., 2008. Effect of organic and nitrogen fertilizers on potato growth and production (*Solanum tuberosum* L.). Asian J. Appl. Sci., 6(1): 136-145. <https://doi.org/10.32649/ajas.2008.32228>
- Ibrahim, M.M. 2015. Response of Washington Navel Orange Trees to Calcium Chloride Foliar Application. Egyptian J. Hortic., 42: 169-179. <https://doi.org/10.21608/ejoh.2015.1283>
- Hesse, P.R. and P.R. Hesse, 1971. A textbook of soil chemical analysis.
- John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. Soil Sci., 109(4): 214-220. <https://doi.org/10.1097/00010694-197004000-00002>
- Joslyn, M.A., 1970. Physical, chemical, and instrumental methods of analysis. Methods in food analysis.
- Khripach, V.A., V.N. Zhabinskii, R.P. Litvinovskaya, S. Hayat and A. Ahmad. 2010. Brassinosteroids: A class of plant hormone. Springer Sci. Bus. Media, pp. 375-395. https://doi.org/10.1007/978-94-007-0189-2_14
- Lateef, S.M., A.J. Abbas and M.R. Majeed. 2023. Effect of brassinolide and chitosan on vegetative growth and some qualitative parameters of local and blood orange fruits. *Citrus sinensis* L. IOP Conf. Ser. Earth Environ. Sci., 1158(4): 042070. IOP Publishing. <https://doi.org/10.1088/1755-1315/1158/4/042070>
- Munson, R.D., 2018. Soil fertility, fertilizers, and plant nutrition. In Handbook of soils and climate in agriculture, 269-294: CRC Press. <https://doi.org/10.1201/9781351073073-7>
- Nardi, S., P. Carletti, D. Pizzeghello and A. Muscolo. 2009. Biological activities of humic substances. Biophysico-chemical processes involving natural nonliving organic matter in environmental systems. Wiley, 2(1): 305-339. <https://doi.org/10.1002/9780470494950.ch8>
- Novamsky, I., R. Van Eck, C.H. Van Schouwenburg, and I. Walinga. 1974. Total nitrogen determination in plant material by means of the indophenol-blue method. Netherlands J. Agric. Sci., 22(1): 3-5.
- Ross, J.J. and L.J. Quittenden. 2016. Interaction between brassinosteroids gibberellins: Synthesis or signalling? Plant Cell, 28: 829-832. <https://doi.org/10.1105/tpc.15.00917>
- Tahir, S., S. Zafar, M.Y. Ashraf, S. Perveen and S. Mahmood. 2022. Assessment of maize genotypes for drought tolerance using physiological indices. Pak. J. Bot., 55(3): 1-10. [https://doi.org/10.30848/PJB2023-3\(8\)](https://doi.org/10.30848/PJB2023-3(8))
- Tahira, A., S. Ahmad, M. Ashraf, M.A. Shahid, M. Yasin, R.M. Balal, S.A. Abbas. 2013. Effect of humic and application at different growth stages of kinnow mandarin (*Citrus reticulata* Blanco) on the basis of physio-biochemical and reproductive responses. Am. J. Bus., 1(1): 014-020. <https://doi.org/10.15413/ajb.2012.0106>
- Tang, J., Z. Han and J. Chai. 2016. What are brassinosteroids and how do they act in plants?

- BMC Biol., 14: 1-5.
- Zafar, S., S. Khan, D. Ibrar, M.K. Khan, Z. Hasnain, K. Mehmood, A. Rais, S. Gul, S. Irshad and M. Nawaz. 2024. Application of zinc nanoparticles as seed priming agent improves growth and yield of wheat seedlings grown under salinity stress by enhanced antioxidants activities and gas exchange attributes. Cereal Res. Commun., pp. 1-14. <https://doi.org/10.1007/s42976-024-00499-2>
- Zafar, S., M. Ashraf, M. Yasin, K.N. Abida and J. Hussain. 2015. Evaluation of wheat genotypes for salinity tolerance using physiological indices as screening tool. Pak. J. Bot., 47(2): 397-405.
- Zafar, S., Z. Hasnain, S. Anwar, S. Perveen, A. Noman, N. Iqbal and M. Ali. 2019. Influence of melatonin on antioxidant defense system and yield of wheat (*Triticum aestivum* L.) genotypes under saline condition. Pak. J. Bot., 51(4): 1987-1994. [https://doi.org/10.30848/PJB2019-6\(5\)](https://doi.org/10.30848/PJB2019-6(5))
- Zuhair, H., S. Zafar, S. Usman, L. Zhang and H.O. Elansary. 2023. Elucidating role of melatonin foliar spray in ameliorating adverse effects of drought stress on growth and physio-biochemical attributes of *Brassica rapa* plants. Sci. Hortic., 321: 112336. <https://doi.org/10.1016/j.scienta.2023.112336>