Role of humic acid and spraying with the growth regulator CPPU in improving the vegetative traits of some citrus rootstocks

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Abstract

The experiment was carried out in the shade of the Department of Horticulture and Landscape / College of Agriculture / University of Kerbala for the period from (1/3/2024) to (1/11/2024). To evaluate the response of citrus rootstocks to soil-applied humic acid and foliar application of CPPU on vegetative growth traits of six-month-old seedlings, a 3×3×3 factorial experiment was conducted using a completely randomized block design (CRBD) with three replicates. Treatments included humic acid (0, 3 and 6 g L^{-1}), CPPU (0, 5 and 10 mg L^{-1}), and three citrus rootstocks (Rangpur, Macrophylla, Sour Orange). The humic acid soil addition treatment 6 g L^{-1} significantly outperformed the control treatment, recording the highest averages for plant height (37.04 cm), stem diameter (5.075 mm), number of leaves (197.74 leaves $plant^{-1}$), number of branches (4.330 branches $plant^{-1}$), leaf area (422.86 cm^2), dry weight of the vegetative system (13.110 g), and dry weight of the root system (5.755 g). The spraying CPPU at 10 mg L^{-1} also showed superiority, achieving notable increases in the same traits an averages of (32.40 cm, 5.036 mm, 197.07 leaves $plant^{-1}$, 4.354 branches $plant^{-1}$, 397.15 cm^2, 12.633 g, 5.637 g). The results also showed significant differences between the rootstocks, with the Rangpur rootstock achieving the highest values in most of the traits under study. Moreover, the combined treatments, particularly the triple interaction (Rangpur + humic acid at 6 g L^{-1} + CPPU 10 mg L^{-1}), significantly enhanced several vegetative growth traits.

Keywords: Humic acid , Growth regulator CPPU ,Citrus rootstocks.

Introduction

Citrus trees belong to the Rutaceae family, which has several genera, the most important of which is the economic genus Citrus. Citrus is widespread in tropical and subtropical regions [1]. Citrus fruits are characterized by containing oil glands in most of their parts, which give them a distinctive smell that distinguishes them from other fruit trees. In addition, they are of great importance among fruit trees due to their nutritional, economic, medicinal and aesthetic value. It contains many minerals such as calcium, phosphorus, potassium, iron and other elements. In addition, it is rich in vitamins such as vitamin C, vitamin A, B1, B2, and it is also a source of dietary fiber [2]. The low citrus production in Iraq compared to global production may be due to the use of unsuitable stocks for propagating various citrus species and the excessive use of chemical fertilizers. Therefore, the most common and recently introduced stocks in Iraq are sour Orange, Rangpur and Macrophylla, which are characterized by unique growth characteristics, including their compatibility with most citrus varieties and their resistance to unfavorable environmental conditions, The Sour Orange rootstock is characterized by a semi-short root and resistance to root rot disease. It is compatible with most commercial varieties, but it is susceptible to infection by nematodes and tristeza disease[3,4]. The Rangpur rootstock is characterized by its tolerance to salinity, resistance to citrus tristeza, succulence in loamy and light soils, and resistance to nematodes. It is a hybrid rootstock resulting from the crossbreeding of sour lemon, while the Macrophylla rootstock is tolerant of calcareous and sandy soils. Grafted varieties are vigorous and highly productive. Originally from the Philippines, it is believed to be a hybrid between the Benzohir and the Adalia lemon due to their similar phenotypic and genetic characteristics. It has smaller leaves compared to the sour Orange[5,6].

Organic fertilizers are essential for improving soil fertility, as they provide a variety of essential nutrients that enhance the activity of microorganisms and improve seedlings' absorption of nutrients, leading to increased seedling growth [7,8]. Among the most prominent of these fertilizers is humic fertilizer, which activates the action of plant enzymes. In addition, it is used as a complementary source of phenols in the early stages, which increases the biological efficiency of the plant and stimulates hanrespiratio, photosynthesis, phosphorus absorption, and cell expansion [9,10,11,12]. It also which contains many nutrients such as nitrogen, phosphorus, iron and amino acids, which work to lower soil pH and thus improve the availability of trace elements, enhance soil biological activity and improve seedling growth, cell elongation and root growth, reducing the pH of the root environment increases nutrient availability because iron is more soluble, and phosphorus is released from insoluble compounds and is more available and efficient. Reducing pH also generally improved nitrogen uptake, particularly the ammonium form, and allowed organic matter decomposition and release of amino acids, rather than being bound as unavailable nutrients typically a moderate pH range (5.5–6.5) is ideal nutrient availability for plants. [13,14,15]. The use of plant growth regulators, especially growth promoters such as auxins, cytokinins and gibberellins, stimulates cell division and elongation. N-(2-Chloro-4-Pyridyl)-N-Phenylurea (CPPU) is one of the most effective cytokinins in stimulating cell division and expansion [16].

Treatment with the growth regulator CPPU is 10-100 times more effective than benzyl adenine (BA), which contributes to achieving good positive results when sprayed on citrus rootstocks, as it leads to physiological effects, including stimulating cell division and increasing cell expansion. It also works to break the apical dominance of the terminal bud, thus stimulating the growth of lateral buds and growth in general [17,18]. Due to the lack of studies on the use of newly introduced citrus rootstocks in Iraq and the addition of humic organic fertilizer and CPPU growth regulator, the study aim to determine the best concentration of humic acid fertilizer and CPPU growth regulator and their interaction in improving the vegetative and root growth characteristics and chemical content of citrus rootstocks in order to produce seedlings suitable for grafting with less time, effort and cost.

Materials and methods

Experiment Site and Implementation

The experiment was conducted on citrus rootstocks in a Saran-covered canopy with 50% shading in the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Karbala, located in Al-Hussainiya District, from March 1, 2024 to November 1, 2024.

Seedling Preparation and Agricultural Operations

Seedlings of (243) citrus rootstocks (Rangpur, Sour Orange, and Macrophylla) were selected, with homogeneous growth, 6 months old, and free of pathogenic pests. The seedlings were planted in 1.25 kg polyethylene bags obtained from the Karbala Citrus Project Station, certified by the Horticulture Department, Ministry of Agriculture, Tuwairij District, Karbala Governorate. On 1/2024, The seedlings were transferred to plastic containers with a capacity of (10 kg). filled with sandy soil mixed with peat moss at a ratio of 1:3. The soil of the containers was sterilized before rotating the seedlings with Beltanol at a concentration of (0.5 ml L^{-1}) as a preventive fungicide. The canopy soil was scraped to a depth of (10 cm) and covered with agricultural nylon to prevent the growth of bushes and weeds and facilitate taking readings for root characteristics later. After that, the containers were randomly distributed within the experimental sectors within the plant canopy. Service operations were also carried out, including irrigation and weeding, and between replicates for all treatments, and equally whenever necessary, until the completion of recording the studied characteristics on (11/1/2024).

Study Factors

The experiment included three factors

The first factor was the addition of humic acid using the fertilization method at three concentrations (0, 3, and 6 g L^{-1}). Humic acid fertilizer was added on March 15, 2024, in four batches, 30 days apart. The second factor involved spraying with the growth regulator CPPU at three concentrations (0, 5, and 10 mg L^{-1}). Seedlings were sprayed from April 1, 2024, to May 1, 2024, at a rate of one spray every 30 days. The third factor involved three citrus rootstocks (orange bitter, rangpur, and Macrophylla).

Experimental Design and Statistical Analysis

A 3×3×3 factorial experiment was conducted using a randomized complete block design (RCBD) with three replicates. Each replicate included (27) treatments, with three seedlings per experimental unit, resulting in a total of 243 seedlings. The results were analyzed using an ANOVA table using the Genstat 2007 statistical analysis program, and the averages were compared using the least significant difference (LSD) at a probability level of 0.05[١٩].

Data Collection

Average increase in seedling height (cm)

The increase in plant height was measured by finding the differences in stem height before and after the experimental treatments, using a tape measure graduated from the soil surface to the highest peak of the main stem. The average was extracted for each experimental unit for the treatments used in the study and for each replicate.

Average increase in seedling diameter (mm)

Stem diameter increase was measured before and after the experimental treatments at a distance of 2 cm from the surface using a Vernier digital caliper. The average was used for each experimental unit for the treatments used in the study and for each replicate.

Number of leaves (leaf plant^{-1})

The total number of leaves per seedling was calculated at the end of the experiment in each experimental unit and in each replicate.

Number of branches (branch plant^{-1})

The total number of branches per seedling was calculated at the end of the experiment in each experimental unit and in each replicate.

Leaf area (cm^2)

Leaf area was calculated using the Image program in Windows 7. By multiplying the area of one leaf by the number of leaves per plant, the leaf area per plant was calculated [20,21].

Dry weight of the shoot system (g)

The dry weight of the vegetative system (g) was calculated by separating the vegetative system from the root system. They were then washed with water to remove dust and impurities, and the fresh weight was calculated using a sensitive electric balance. The samples were then placed in separate paper bags and dried in an electric oven at a temperature of (70°C) for (48) hours until the dry weight was established. The dry weight was measured using a sensitive electric balance [22].

Dry weight of the root system (g)

The dry weight of the root system (g) was calculated by removing the seedlings from the anvils after the end of the experiment for each experimental unit of the experimental factors, separating the vegetative system from the root system from the swollen crown area, washing the roots with water, and taking their wet weight. They were placed in perforated paper bags in an electric oven at a temperature of (70°C) for (48) hours until the dry weight was fixed, as calculated by the dry weight scale

Results and Discussion

Plant height

Table (1) shows a significant effect of humic acid, growth regulator and rootstock on the average increase in plant height. Humic acid at a concentration of (6 g L^{-1}) recorded a significant superiority in the average increase in plant height, reaching (37.04 cm), compared to the control treatment, which recorded the lowest average increase in plant height, reaching (19.68cm). Averagewhile, the spraying treatment with CPPU growth regulator at a concentration of (10 mg L^{-1}) showed a significant superiority in the average increase in plant height, reaching (32.40 cm), compared to the control treatment, which gave the lowest average, reaching (24.33 cm). The orange rootstock also outperformed, giving the highest average increase in plant height, reaching (33.31cm), compared to the Macrophylla rootstock, which gave the lowest average plant height, reaching (21.69cm). The results Table(1) show that the two-way interaction treatment (humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) achieved the highest interaction (41.54 cm), while the control treatment for both factors recorded the lowest interaction (15.26cm). The interaction treatment (Sour orange rootstock + humic acid at 6 g L^{-1}) also achieved the highest interaction (42.02 cm), which did not differ significantly from the treatment (Rangpur rootstock + humic at a concentration of 6 g L^{-1}) which reached (40.44 cm) compared to the other treatments, while the treatment (Macrophylla rootstock + humic at 0 g L^{-1}) recorded the lowest average increase in plant height of (13.41 cm). The results also indicated the presence of a significant interaction between the rootstock and the growth regulator CPPU, as the interaction (Sour orange rootstock + CPPU at 10 mg L^{-1}) was outperformed, giving the highest interaction with an average of (38.89 cm), while the interaction treatment (macrophylla + CPPU at 0 mg L^{-1}) gave the lowest average of (18.98cm).

The results of the triple interaction between the study factors showed a significant effect on the average stem height, as the triple interaction treatment (Sour orange rootstock + humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) was outperformed, giving the highest average of (48.33cm) compared to the triple interaction treatment (Macrophylla rootstock + humic acid at 0g L^{-1} + CPPU at 0 mg L^{-1}) which gave the lowest average of (10.22cm).

Table (1): Effect of humic acid, growth regulator CPPU, rootstock and their interactions on the of average plant height(cm).

Rootstock	**Humic acid (g L^{-1})**	**CPPU (mg L^{-1})**			**Rootstock × Humic acid**
		0	**5**	**10**	
Sour orange	0	19.33	26.44	29.83	25.20
	3	28.06	31.56	38.50	32.70
	6	34.44	43.28	48.33	42.02
Rangpur	0	16.22	20.78	24.28	20.43
	3	26.78	29.61	33.11	29.83
	6	37.22	39.89	44.22	40.44
Macrophylla	0	10.22	13.78	16.22	13.41
	3	20.67	23.33	25.06	23.02
	6	26.06	27.83	32.06	28.65
L.S.D $_{0.05}$		5.174			2.987
Humic acid × CPPU					Average (Humic acid)
0		15.26	20.33	23.44	19.68
3		25.17	28.17	32.22	28.52
6		32.57	37.00	41.54	37.04
L.S.D $_{0.05}$		2.987			1.725
Rootstock × CPPU					Average (Rootstock)
Sour orange		27.28	33.78	38.89	33.31
Rangpur		26.74	30.09	33.87	30.23
Macrophylla		18.98	21.65	24.44	21.69
L.S.D $_{0.05}$		2.987			1.725
Average (CPPU)		٢٤,٣٣	28.50	٣٢,٤٠	
L.S.D $_{0.05}$		1.725			

Stem diameter

From Table (2), we note that humic fertilizer at a concentration of (6 g L^{-1}) significantly outperformed the other treatments in the stem diameter increase trait, reaching (5.075 mm), recorded the lowest average of (٣،٧٦٢ mm) . The spraying treatment with the growth regulator CPPU at a concentration of (10 mg L^{-1}) also achieved the highest average stem diameter increase, reaching (5.036 mm), compared to the control treatment, which gave the lowest average stem diameter increase, reaching (3.456 mm). The Rangpur rootstock also significantly outperformed the other rootstocks in the average stem diameter increase, reaching (4.963 mm), compared to the Macrophylla rootstock, which gave the lowest average stem diameter increase, reaching (3.872 mm). The data presented in table (2) revealed a significant interaction between humic acid and the growth regulator, as the two-way interaction treatment (humic acid 6 g L^{-1} + CPPU 10 mg L^{-1}) recorded the highest average of (5.972 mm) compared to the control treatment for both factors (humic acid 0 g L^{-1} + CPPU 0 mg L^{-1}), which recorded the lowest average of (2.787 mm). The results also indicated a significant interaction between citrus rootstocks and humic acid concentrations, where the interaction (Rangpur rootstock + humic acid at 6 g L^{-1}) achieved the highest average of (6.003 mm), while the interaction (Macrophylla rootstock + humic acid at 0 g L^{-1}) recorded the lowest average of (3.499 mm). Furthermore, a significant interaction was found between rootstocks and the growth regulator, as the interaction (Rangpur rootstock + CPPU 10 mg L^{-1}) recorded the highest average of (5.840 mm), while the interaction (Sour Orange rootstock + CPPU 0 mg L^{-1}) gave the lowest average of (٣,٢٦٣ mm).

From the results presented in Table (2), a significant triple interaction was observed among the experimental factors, as the interaction (Rangpur rootstock + humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) excelled by achieving the highest interaction with a average of (٧،١٢٥mm), while the interaction (sour orange rootstock + humic acid at 0 g L^{-1} + CPPU at 0 mg L^{-1}) recorded the lowest average of (٢،٥٧٢mm).

Table (2): Effect of humic acid, growth regulator CPPU, rootstock and their interactions on the stem diameter(mm).

Rootstock	Humic acid (g L^{-1})	CPPU (mg L^{-1})			Rootstock × Humic acid
		0	5	10	
Sour orange	0	٢,٥٧٢	٤,١٣٠	4.367	٣,٦٩٠
	3	٣,٥٥٢	٤,٢١٩	4.464	٤,٠٧٨
	6	٣,٦٦٥	٥,٠٧٨	6.048	٤,٩٣٠
Rangpur	0	2.958	4.448	4.889	٤,٠٩٨
	3	3.687	٥,١٧٢	٥,٥٠٥	٤,٧٨٨
	6	4.698	٦,١٨٦	٧,١٢٥	٦,٠٠٣
Macrophylla	0	2.831	٣,٧٩٦	٣,٨٧٠	٣,٤٩٩
	3	3.559	٣,٥٩٧	٤,٣١٨	٣,٨٢٤
	6	3.584	٤,٥٤٩	٤,٧٤٢	٤,٢٩٢
L.S.D $_{0.05}$		0.2874			٠,١٦٥٩

Humic acid × CPPU				Average (Humic acid)
0	2.787	٤,١٢٥	٤,٣٧٥	٣,٧٦٢
3	3.599	٤,٣٢٩	٤,٧٦٢	٤,٢٣٠
6	3.982	٥,٢٧١	٥,٩٧٢	٥,٠٧٥
L.S.D $_{0.05}$	0.1659			0.0958
Rootstock × CPPU				Average (Rootstock)
Sour orange	٣,٢٦٣	٤,٤٧٦	4.960	٤,٢٣٣
Rangpur	3.781	٥,٢٦٩	٥,٨٤٠	٤,٩٦٣
Macrophylla	٣,٣٢٥	٣,٩٨١	٤,٣١٠	٣,٨٧٢
L.S.D $_{0.05}$	0.1659			0.0958
Average (CPPU)	٣,٤٥٦	٤,٥٧٥	٥,٠٣٦	
L.S.D $_{0.05}$	0.0958			

Number of branches

The results presented in Table (3) show that humic acid had a significant effect on the number of branches, as the level of (6 g L^{-1}) was superior, recording the highest average number of branches, reaching (4.330 branches $plant^{-1}$), compared to the control treatment, which gave the lowest average number of branches, reaching (3.359 branches $plant^{-1}$). The spraying treatment with a growth regulator at a concentration of (10 mg L^{-1}) was superior, giving the highest average number of branches, reaching (4.354 branches $plant^{-1}$), compared to the control treatment, which gave the lowest average number of branches, reaching (3.043 branches $plant^{-1}$). As for the rootstock, it had a significant effect on the number of branches, as the Rangpur rootstock outperformed it in the number of branches, reaching (4.123 branches $plant^{-1}$), compared to the Macrophylla rootstock, which gave the lowest average number of branches, reaching (3.526 branches $plant^{-1}$).

The results in Table (3) show that the two-way interaction treatment (humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) achieved the highest interaction (٤,٨٩٢ branches $plant^{-1}$), while the control treatment for both factors recorded the lowest interaction (٢,٦٦٢ branches $plant^{-1}$). The interaction treatment (Rangpur rootstock + humic acid at 6 g L^{-1}) also achieved the highest interaction (4.669 branches plant $^{-1}$), while the interaction (Macrophylla + humic acid at 0 g L^{-1}) recorded the lowest interaction (3.114 branches plant $^{-1}$). The results also indicated the presence of a significant interaction between the rootstock and the growth regulator CPPU, as the interaction (Rangpur rootstock + CPPU at 10 mg L^{-1}) was outperformed, giving the highest interaction with an average of (4.634 branches plant $^{-1}$), while the interaction treatment (Macrophylla + CPPU at 0 mg L^{-1}) gave the lowest average of (2.874 branches $plants^{-1}$).

The results also showed that the triple interaction among the study factors had a significant effect, as the treatment (Rangpur rootstock + humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) outperformed by recording the highest average of (٥،٢٣٢ branches plant $^{-1}$), while the interaction (Sour Orange rootstock + humic acid at 0 g L^{-1} + CPPU at 0 mg L^{-1}) recorded the lowest average of (٢،٤٣٩ branches plant $^{-1}$).

Table (3): Effect of humic acid, growth regulator CPPU, rootstock and their interactions on the number of branches (branch plant $^{-1}$).

Rootstock	Humic acid (g L^{-1})	CPPU (mg L^{-1})			Rootstock × Humic acid
		0	5	10	
Sour orange	0	٢,٤٣٩	٣,٤٤٦	٤,١٢١	٣,٣٣٥
	3	٢,٨٨٩	٤,١١٠	٤,٣٣٣	٣,٧٧٧
	6	٣,٣٣٤	٤,٥٥٥	٤,٨٨٦	٤,٢٥٨
Rangpur	0	٢,٨٨٣	٣,٧٧٥	٤,٢٢٣	٣,٦٢٧
	3	٣,٤٤٣	٤,٣٣٣	٤,٤٤٦	٤,٠٧٤
	6	٣,٧٧٥	٥,٠٠٠	٥,٢٣٢	٤,٦٦٩
Macrophylla	0	٢,٦٦٤	٣,١١٢	٣,٥٦٧	٣,١١٤
	3	٢,٧٧٨	٣,٦٠٧	٣,٨١٧	٣,٤٠١
	6	٣,١٨٠	٤,٤٥٠	٤,٥٥٧	٤,٠٦٢
L.S.D $_{0.05}$		٠,١٠٥٣			0.0608
Humic acid × CPPU					Average (Humic acid)
0		٢,٦٦٢	٣,٤٤٤	٣,٩٧١	٣,٣٥٩
3		٣,٠٣٧	٤,٠١٧	٤,١٩٩	٣,٧٥١
6		٣,٤٣٠	٤,٦٦٨	٤,٨٩٢	٤,٣٣٠
L.S.D $_{0.05}$		0.0608			0.0351
Rootstock × CPPU					Average (Rootstock)
Sour orange		٢,٨٨٧	٤,٠٣٧	٤,٤٤٧	٣,٧٩٠
Rangpur		٣,٣٦٧	٤,٣٦٩	٤,٦٣٤	٤,١٢٣
Macrophylla		٢,٨٧٤	٣,٧٢٣	٣,٩٨٠	٣,٥٢٦
L.S.D $_{0.05}$		0.0608			0.0351
Average (CPPU)		٣,٠٤٣	٤,٠٤٣	٤,٣٥٤	
L.S.D $_{0.05}$		0.0351			

Number of leaves

The results in Table (4) showed that the treatment with 6 g L^{-1} humic acid fertilizer was superior, as it recorded the highest average number of leaves, reaching (197.74 leaves plant $^{-1}$), while the control treatment recorded the lowest average number of leaves, reaching (149.20 leaves plant^{-1}). The treatment with 10 mg L^{-1} growth regulator spray also outperformed, producing the highest average number of leaves, reaching (197.07 leaves plant^{-1}), compared to the control treatment, which recorded the lowest average number of leaves, reaching(139.10 leaves plant^{-1}). The results in Table (4) also showed that the Rangpur rootstock outperformed the other rootstocks, producing the highest average number of leaves, reaching (223.96 leaves plant^{-1}), compared to the Macrophylla rootstock, which produced the lowest average number of leaves, reaching (119.89 leaves plant^{-1}). The results showed a significant interaction between humic acid and growth regulator, as the interaction (humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) was outperformed, giving the highest interaction with an average of (229.85 leaves plant^{-1}), while the lowest interaction was in the control treatment for both factors with an average of (116.22 leaves plant^{-1}). The results of the same in table also showed a significant two-way interaction between the rootstock and humic acid, as the Rangpur rootstock with humic acid (6g L^{-1}) recorded the highest interaction with an average of (261.26 leaves plant^{-1}), while the Macrophylla rootstock with the control treatment of humic acid (0g L^{-1}) recorded the lowest interaction of (96.37 leaves plant^{-1}). It was noted from the results in Table (4) that the interaction between the rootstock and the growth regulator had a significant effect on number of leaves, reaching, as the highest interaction was with the Rangpur rootstock and the growth regulator at a concentration of (10 mg L^{-1}) with an average of (259.19 leaves plant^{-1}), while the lowest interaction was with the Macrophylla rootstock with the control treatment of the growth regulator (0 mg L^{-1}) which recorded an average of (95.96 leaves plant^{-1}).The results also showed that the triple interaction among the study factors had a significant effect, as the treatment (Rangpur rootstock + humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) outperformed by recording the highest average of (٢٩١,٣٣ leaves plant), while the interaction (Macrophylla rootstock + humic acid at 0 g L^{-1} + CPPU at 0 mg L^{-1}) recorded the lowest average of (٨٥,٣٣ leaves plant^{-1}).

Table (4): Effect of humic acid, growth regulator CPPU, rootstock and their interactions on the number of leaves (leaves plant^{-1}).

Rootstock	Humic acid (g L^{-1})	CPPU (mg L^{-1})			Rootstock × Humic acid
		0	5	10	
Sour orange	0	١٢٩,٦٧	١٦٣,٠٠	١٦٥,١١	١٥٢,٥٩
	3	١٥٠,٦٦	١٧٣,٣٣	١٨٥,٤٥	١٦٩,٨١
	6	١٥٥,٠٠	١٩٠,٠٠	٢٠٧,١١	١٨٤,٠٤
Rangpur	0	١٣٣,٤٤	٢٢٢,٨٩	٢٣٩,٥٦	١٩٨,٦٣
	3	١٩١,٣٤	١٩٨,٠٠	٢٤٦,٦٧	٢١٢,٠٠
	6	٢٠٣,٨٩	٢٨٨,٥٦	٢٩١,٣٣	٢٦١,٢٦
Macrophylla	0	٨٥,٥٥	٩٣,٧٨	١٠٩,٧٨	٩٦,٣٧

	3	٨٨,٣٣	١٢٠,٢٢	١٣٧,٥٦	١١٥,٣٧
	6	١١٤,٠٠	١٣٨,٦٧	١٩١,١١	١٤٧,٩٣
L.S.D $_{0.05}$		٤,٠٨٣			٢,٣٥٧
Humic acid × CPPU					Average (Humic acid)
0		١١٦,٢٢	١٥٩,٨٩	١٧١,٤٨	149.20
3		١٤٣,٤٤	١٦٣,٨٥	١٨٩,٨٩	١٦٥,٧٣
6		١٥٧,٦٣	٢٠٥,٧٤	٢٢٩,٨٥	197.74
L.S.D $_{0.05}$		٢,٣٥٧			1.361
Rootstock × CPPU					Average (Rootstock)
Sour orange		١٤٥,١١	١٧٥,٤٤	١٨٥,٨٩	168.81
Rangpur		١٧٦,٢٢	٢٣٦,٤٨	٢٥٩,١٩	223.96
Macrophylla		٩٥,٩٦	١١٧,٥٦	١٤٦,١٥	119.89
L.S.D $_{0.05}$		٢,٣٥٧			١,٣٦١
Average (CPPU)		139.10	١٧٦,٤٩	١٩٧,٠٧	
L.S.D $_{0.05}$		1.361			

Leaf area

The results in Table (5) showed that the humic acid fertilizer treatment at a concentration of (6 g L^{-1}) was superior, recording the highest average leaf area of (422.86 cm^2) compared to the control treatment which recorded the lowest average of(285.15). As for the spraying treatment with growth regulator at a concentration of (10 mg L^{-1}), it recorded the highest average leaf area of (397.15 cm^2) compared to the control treatment, which gave the lowest average of(305.48 cm^2). The results also showed that the Rangpur rootstock was superior among the three rootstocks in leaf area of (428.02 cm^2) .while the Macrophylla rootstock recorded the lowest average of(225.85 cm^2). As for the two-way interaction between humic acid and the growth regulator (CPPU), it was observed that the treatment (humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) achieved the highest interaction, with a average of (479.62 cm^2), compared to the control treatment, which recorded the lowest interaction average of (244.58 cm^2).The results also indicated that the two-way interaction between the rootstock and humic acid (Rangpur rootstock + humic acid at 6 g L^{-1}) recorded the highest interaction average of (525.13 cm^2), while it was observed that the Macrophylla rootstock with humic acid at (0 g L^{-1}) recorded the lowest interaction average of (195.59 cm^2). The results of the statistical analysis in Table(5) also indicated that the two-way interaction between the rootstock and the growth regulator CPPU significantly affected the leaf area , as the treatment with the growth regulator at a concentration (10 mg L^{-1}) with the Rangpur rootstock recorded the highest interaction of (474.03 cm^2), while the

Macrophylla rootstock with the growth regulator at a concentration (0 mg L^{-1}) recorded the lowest interaction of (194.98cm^2).

The results of the triple interaction between the study factors also indicated a significant effect on the average leaf area , as the triple interaction treatment (Rangpur rootstock + humic acid 6 g L^{-1} + CPPU 10 mg L^{-1}) was outperformed, giving the highest average of (٥٨٣,٦٣ cm^2) compared to the treatment (Macrophylla rootstock + humic acid 0 g L-1 + CPPU 0 mg L^{-1}), which gave the lowest average of (١٦٣,٠٧ cm^2).

Table (5): Effect of humic acid, growth regulator CPPU, rootstock and their interactions on the leaf area (cm^2)

Rootstock	Humic acid (g L^{-1})	CPPU (mg L^{-1})			Rootstock × Humic acid
		0	5	10	
Sour orange	0	٢٧٧,٣٥	٣١٧,٧٨	٣٦٣,٦٠	٣١٩,٥٨
	3	٣٤٠,٨٧	٤٠٣,٧٧	٤٣٨,٨٥	٣٩٤,٥٠
	6	٤١٥,٨٦	٤٩٤,٣٣	٥٦١,٨١	٤٩٠,٦٧
Rangpur	0	٢٩٣,٣١	٣٥٣,٧٦	٣٧٣,٨١	٣٤٠,٢٩
	3	٣٧٣,١٨	٤١٨,٠٧	٤٦٤,٦٣	٤١٨,٦٣
	6	٤٦٣,٨٤	٥٢٧,٩١	٥٨٣,٦٣	٥٢٥,١٣
Macrophylla	0	١٦٣,٠٧	١٩٠,٩٢	٢٣٢,٧٩	١٩٥,٥٩
	3	٢٠٢,٩٣	٢٢٢,٨٤	٢٦١,٧٦	٢٢٩,١٧
	6	٢١٨,٩٤	٢٤٥,٩٨	٢٩٣,٤٣	٢٥٢,٧٨
L.S.D $_{0.05}$		٩,٣٦٣			٥,٤٠٦
Humic acid × CPPU					Average (Humic acid)
0		٢٤٤,٥٨	٢٨٧,٤٩	٣٢٣,٤٠	٢٨٥,١٥
3		٣٠٥,٦٦	٣٤٨,٢٣	٣٨٨,٤٢	٣٤٧,٤٣
6		٣٦٦,٢١	٤٢٢,٧٤	٤٧٩,٦٢	٤٢٢,٨٦
L.S.D $_{0.05}$		5.406			3.121
Rootstock × CPPU					Average (Rootstock)
Sour orange		٣٤٤,٧٠	٤٠٥,٢٩	٤٥٤,٧٥	٤٠١,٥٨
Rangpur		٣٧٦,٧٨	٤٣٣,٢٥	٤٧٤,٠٣	٤٢٨,٠٢
Macrophylla		١٩٤,٩٨	٢١٩,٩١	٢٦٢,٦٦	٢٢٥,٨٥
L.S.D $_{0.05}$		٥,٤٠٦			٣,١٢١
Average (CPPU)		٣٠٥,٤٨	٣٥٢,٨٢	٣٩٧,١٥	
L.S.D $_{0.05}$		3.121			

Dry weight of the shoot system

The statistical data in Table (6) indicate that the addition of humic fertilizer at a concentration of (6 g L^{-1}) had a significant effect on the dry weight of the shoot system, as the treatment gave the highest average dry weight of the vegetative system, reaching (13.110 g), while the control treatment gave the lowest average dry weight of the vegetative system, reaching (8.642 g). The treatment of spraying with growth regulator at a concentration of (10 mg L^{-1}) also outperformed by recording the highest average dry weight of the root system, reaching (12.633 g), compared to the control treatment, which achieved the lowest average of (٨,٣٦٧). The results of Table (6) show that the Rangpur rootstock achieved the highest average dry weight of the vegetative system, reaching (13.700g), compared to the Macrophylla rootstock, which gave the lowest average dry weight of the vegetative system, reaching (8.562g) The results in Table(6) indicated a significant interaction between humic acid and the growth regulator (CPPU), where the interaction treatment (humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) recorded the highest average of (15.084 g) compared to the control treatment for both factors (humic acid at 0 g L^{-1} + CPPU at 0 mg L^{-1}), which gave the lowest average of (6.804 g). The results also revealed a significant interaction between citrus rootstocks and humic acid concentrations, as the interaction (Rangpur rootstock + humic acid at 6 g L^{-1}) recorded the highest average of (16.277 g), whereas the interaction (Macrophylla rootstock + humic acid at 0 g L^{-1}) gave the lowest average of (7.157g). The results further showed a significant interaction between citrus rootstocks and foliar application of CPPU, where the interaction (Rangpur rootstock + CPPU at 10 mg L^{-1}) achieved the highest average of (15.768 g), while the interaction (Sour Orange rootstock + CPPU at 0 mg L^{-1}) recorded the lowest average of (6.063g).

The data presented in Table (6) indicated a significant triple interaction among the experimental factors. The triple interaction (Rangpur rootstock + humic acid at 6 g L-1 + CPPU at 10 mg L-1) recorded the highest average of (١٨,٦٧٠g), while the triple interaction (Sour Orange rootstock + humic acid at 0 g L^{-1} + CPPU at 0 mg L^{-1}) gave the lowest average of (٣,٣٤٣ g).

Table (6): Effect of humic acid, growth regulator CPPU, rootstock and their interactions on the dry weight of the shoot system (g).

Rootstock	Humic acid (g L^{-1})	CPPU (mg L^{-1})			Rootstock × Humic acid
		0	5	10	
Sour orange	0	٣,٣٤٣	٩,٥٥٣	٩,٧٣٠	٧,٥٤٢
	3	٣,٦٨٧	١١,٢٦٠	١٢,٥٩٠	٩,١٧٩
	6	١١,١٦٠	١٣,٠٢٤	١٤,٧٤٣	١٢,٩٧٦
Rangpur	0	١٠,٢٩٠	١٠,٨٨٠	١٢,٥١٣	١١,٢٢٨
	3	١١,٢٨٠	١٣,٣٩٠	١٦,١٢٠	١٣,٥٩٧

	6	١٣,٤٠٠	١٦,٧٦٠	١٨,٦٧٠	١٦,٢٧٧
Macrophylla	0	٦,٧٨٠	٧,٠٩٠	٧,٦٠٠	٧,١٥٧
	3	٧,١٩٠	٨,٢٨٠	٩,٨٩٠	٨,٤٥٣
	6	٨,١٧٠	١٠,٢٢٠	١١,٨٤٠	١٠,٠٧٧
L.S.D 0.05		٠,٧٣٢٢			٠,٤٢٢٧
Humic acid × CPPU					Average (Humic acid)
0		٦,٨٠٤	٩,١٧٤	٩,٩٤٨	٨,٦٤٢
3		٧,٣٨٦	١٠,٩٧٧	١٢,٨٦٧	١٠,٤١٠
6		١٠,٩١٠	١٣,٣٣٥	١٥,٠٨٤	١٣,١١٠
L.S.D 0.05		٠,٤٢٢٧			٠,٢٤٤١
Rootstock × CPPU					Average (Rootstock)
Sour orange		٦,٠٦٣	١١,٢٧٩	١٢,٣٥٤	٩,٨٩٩
Rangpur		١١,٦٥٧	١٣,٦٧٧	١٥,٧٦٨	١٣,٧٠٠
Macrophylla		٧,٣٨٠	٨,٥٣٠	٩,٧٧٧	٨,٥٦٢
L.S.D 0.05		٠,٤٢٢٧			٠,٢٤٤١
Average (CPPU)		٨,٣٦٧	١١,١٦٢	١٢,٦٣٣	
L.S.D 0.05		٠,٢٤٤١			

Dry weight of the root system

The results in Table (7) showed that the treatment of adding humic fertilizer at a concentration of (6 g L^{-1}) was superior, as the treatment recorded the highest average dry weight of the root system, amounting to (5.755 g), while the control treatment gave the lowest average dry weight of the root system, amounting to (4.127 g). The treatment of spraying with a growth regulator at a concentration of (10 mg L^{-1}) also outperformed, recording the highest average dry weight of the root system, amounting to (5.637 g), compared to the rest of the control treatments, which gave the lowest average dry weight of the root system, amounting to (4.207 g). The statistical analysis in Table (7) also showed that the Rangpur rootstock outperformed the other rootstocks, as it gave the highest average dry weight of the root system, amounting to (5.807 g), compared to the Macrophylla rootstock, which gave the lowest average dry weight, amounting to (3.878 g). As for the two-way interaction between humic acid and the growth regulator (CPPU), it was observed that the treatment (humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) achieved the highest interaction, with a average of (6.554 g), compared to the control treatment, which recorded the lowest interaction average of (3.602 g).The results also indicated that the two-way interaction between the rootstock and humic acid (Rangpur rootstock + humic acid at 6 g L^{-1}) recorded the highest interaction average of (6.999 g), while it was observed that the Macrophylla rootstock with humic acid at (0 g L^{-1}) recorded the lowest interaction average of (3.324 g). The results of the statistical analysis in Table(7) also indicated that the two-way interaction between the rootstock and the growth regulator CPPU significantly affected the root system,as the treatment with the growth regulator at a concentration (10 mg L^{-1}) with the Rangpur rootstock recorded the highest interaction of (7.146 g), while the Macrophylla rootstock with the growth regulator at a concentration (0 mg L^{-1}) recorded the lowest interaction of (3.479 g).

The results in Table (7) showed that the triple interaction treatment (Rangpur rootstock + humic acid at 6 g L^{-1} + CPPU at 10 mg L^{-1}) achieved the highest average of (٨,٥٠٣g), while the triple interaction treatment (Macrophylla rootstock + humic acid at 0 g L^{-1} + CPPU at 0 mg L^{-1}) recorded the lowest average of (٢,٩٨٠g).

Table (7): Effect of humic acid, growth regulator CPPU, rootstock and their interactions on the dry weight of the root system (g).

Rootstock	Humic acid (g L^{-1})	CPPU (mg L^{-1})			Rootstock × Humic acid
		0	5	10	
Sour orange	0	٤,٢٣٣	٤,٧٨٠	٤,٧٨٧	٤,٦٠٠
	3	٤,٥١٧	٥,٤٨٠	٥,٦٢٠	٥,٢٠٦
	6	٥,٢٠٠	٥,٧٣٠	٦,٦٠٠	٥,٨٤٣
Rangpur	0	٣,٥٩٣	٤,٥٨٧	٥,١٩٠	٤,٤٥٧
	3	٤,٦١٣	٥,٥٣٧	٧,٧٤٣	٥,٩٦٤
	6	٥,٢٧٠	٧,٢٢٣	٨,٥٠٣	٦,٩٩٩
Macrophylla	0	٢,٩٨٠	٣,٤٥٣	٣,٥٤٠	٣,٣٢٤
	3	٣,٢٨٣	٤,١٨٧	٤,١٩٠	٣,٨٨٧
	6	٤,١٧٣	٤,٥٣٣	٤,٥٦٠	٤,٤٢٢
$L.S.D_{0.05}$		٠,٣٦٤٢			٠,٢١٠٣
Humic acid × CPPU					Average (Humic acid)
0		٣,٦٠٢	٤,٢٧٣	٤,٥٠٦	٤,١٢٧
3		٤,١٣٨	٥,٠٦٨	٥,٨٥١	٥,٠١٩
6		٤,٨٨١	٥,٨٢٩	٦,٥٥٤	٥,٧٥٥
$L.S.D_{0.05}$		٠,٢١٠٣			٠,١٢١٤
Rootstock × CPPU					Average (Rootstock)
Sour orange		٤,٦٥٠	٥,٣٣٠	٥,٦٦٩	٥,٢١٦
Rangpur		٤,٤٩٢	٥,٧٨٢	٧,١٤٦	٥,٨٠٧
Macrophylla		٣,٤٧٩	٤,٠٥٨	٤,٠٩٧	٣,٨٧٨
$L.S.D_{0.05}$		٠,٢١٠٣			٠,١٢١٤
Average (CPPU)		٤,٢٠٧	٥,٠٥٧	٥,٦٣٧	

L.S.D $_{0.05}$	٠,١٢١٤	

The results in Tables (1-7) demonstrated the superiority of the humic acid soil treatment, as it recorded the highest values in all studied traits, especially at high concentrations (6 g L^{-1}). These results may be attributed to the following reasons: Humic acid improves the chemical and physical properties of the soil, thereby increasing soil fertility, increasing the permeability of cell membranes, and releasing nutrients ready for absorption by plant roots and their transfer to other plant parts, thus positively impacting increased vegetative growth [24, 23]. Furthermore, humic acid treatment provides many nutrients, acting as a reservoir of nutrients. One of these is nitrogen, which plays an important role in increasing cell division. Furthermore, it improves the cation exchange capacity, reduces pH, and increases the availability of nutrients, thus facilitating their absorption by the plant, which positively impacts vegetative growth [25]. The accumulation of nutrients resulting from photosynthesis increases vegetative growth and the formation of a strong vegetative mass. Humic acid plays a similar role to auxins in cell division, as does the presence of amino acids important in the formation of proteins that are affected by enzymes. Consequently, all these positive effects lead to increased growth of vegetative traits [26]. These results are consistent with those reached by [27,28]. in their study of the effect of humic acid on citrus fruits. As we can see from the results in Tables (1-7), the superiority of spraying with the growth regulator CPPU, especially at high concentrations (10 mg L^{-1}), resulted in the highest rates in all studied traits. The positive effect of spraying with the growth regulator CPPU may be due to the growth regulator's role in regulating plant growth and development and stimulating lateral bud growth by breaking the apical dominance induced by auxin [29]. In addition to its role in increasing cell division in the apical meristems [30]. the growth regulator CPPU also activates the activity of the enzyme Protochlorophyllide reductase (NADPH) and stimulates the development of chloroplasts, which is reflected in the increase in carbon metabolism and the accumulation of nutrients, which is positively reflected in the increase in the number of branches, the increase in the diameter of the stem, the height of the plant, the number of leaves, and the increase in the dry weight of the vegetative system and the root system. As for the increase in the leaf area, it may be due to the effect of the growth regulator CPPU by stimulating cell division, which increases the leaf area exposed to light, which leads to an increase in the process of photosynthesis and the accumulation of nutrients that cause an increase in the height of the plant and an increase in the diameter of the stem, the number of branches, the number of leaves, the dry weight of the vegetative system, and the dry weight of the root system [31].The results in tables (2-7) showed that the Rangpur rootstock was superior in all vegetative characteristics except plant height compared to the rest of the rootstocks. This may be due to genetic variation among the rootstocks, the influence of environmental conditions, and the difference in the strength of growth and physiological condition of the rootstocks. This study is consistent with the findings of [32]. in a study conducted on three rootstocks of citrus fruits, stromelia, Volkameriana and sour Orange. This study is also consistent with the findings of [33]. in a study on the Rangpur rootstock.

Based out of our results we see that which in fact did very well was the use of humic acid at 6 g L^{-1} and CPPU at 10 mg L^{-1} which did great for the vegetative growth of citrus seedlings. Also, we saw that Rangpur rootstock did outperform Macrophylla and Sour Orange in most of the indicators. The best performance was seen in the combination of Rangpur rootstock with the highest doses of humic acid and CPPU which had the greatest results in terms of plant height, leaf number, and biomass. Also, what we see is a very strong interactive between the soil and foliar applications which did an excellent job at improving growth. These results put forth that which integrated approaches may be what we use to best improve seedling development in citrus nurseries.

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