



The effect of spraying with Humic acid and Salicylic acid on the growth of mandarin seedlings *Citrus reticulata*

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Received: Oct. 25, 2025	Abstract The experiment was carried out in one of the nurseries located in the Al-Issa area / Kufa district Al-Najaf Governorate for the sea-season 2020-2021, the aim of studying the effect of spraying with humic acid at a concentration (0,5,10) ml L ⁻¹ , and spraying with salicylic acid at a concentration of (0,50,100) mg L ⁻¹ and their interaction in the growth and vegetative characteristics of <i>Citrus reticulata</i> (mandarin) seedlings. The experiment was carried out as a factorial experiment in randomized completely block design (RCBD), with three replications and (9) plants for each replicate. The individual results of the two study factors showed a notable rise in the superiority of humic acid applied at concentration 10 mL L ⁻¹ and salicylic acid supplied at 100 mg L ⁻¹ , in the characteristics studied (Seedling height, stem diameter, number of leaves, leaf area, number of branches, total chlorophyll content of leaves and percentage of carbohydrates in leaves), and the interaction treatment between them at a concentration of (10 ml L ⁻¹ + 100 mg L ⁻¹), in the characteristics studied (Seedling height, stem diameter, number of leaves, leaf area, number of branches, total chlorophyll content of leaves and percentage of carbohydrates in leaves) compared with other treatments and the comparison treatment, reached (31.80 cm, 4.89 mm, 43.47 leaf seedlings ⁻¹ , 11.81 cm ² , 3.19 shoot seedlings ⁻¹ , 126.52 mg 100 g ⁻¹ fresh weight, 12.54%) respectively.
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Introduction

Mandarin *Citrus reticulata*, belong to the Rutaceae family include many genera and species, including Citrus, which is an important evergreen fruit and is widespread

throughout the world due to its adaptation to a wide range of different environmental conditions. It is believed to be native to tropical and subtropical regions between 40 degrees north and south latitude [1], its cultivation also spread in the regions of South-east Asia, China and eastern India before 4000 B.C. [2]. Mandarin is considered one of the most important and widespread citrus fruits in the world, after the orange. It is characterized by its sweet taste, low acidity, and ease of peeling. Its fruits are also distinguished by their high nutritional value, as they contain a high percentage of vitamins (P, B1, B2, and A) also a major source of vitamin C each (100 ml) of juice contains 40–50 mg of this vitamin [3], they are abundant in nutrients and bioactive compounds that offer potential medicinal values [4], the delicate taste of mandarin increases its popularity around the world [5]. Its fruits contain some organic acids, such as citric and malic acid, and are also rich in some mineral salts necessary for building the body, such as potassium, calcium, iron, sulfur, sodium, magnesium, and phosphorus, in addition to their industrial benefits. It is used in food manufacturing; juices are made from it, and volatile aromatic oils are extracted from its peel. As for the remaining peels, pectin (soluble dietary fiber) is extracted from them, which is used in the manufacture of jams and sweets [6, 7], these health attributes of the tangerine fruit are attributed to its richness in phytochemicals, including organic acids, sugars, and amino acids, as well as secondary metabolites such as carotenoids, polyphenols, flavonoids, phenolic acids, and limonoids [8]. The slow growth of citrus seedlings grafted on different rootstocks and the time period it takes to reach the stage suitable for seedlings in a permanent place, it the most important problems that lead to an increase in the costs of seedlings production. Mandarin (*Citrus reticulata*) seedlings are less productive in nurseries because they grow slowly and take a long time to reach marketable size. The effects of humic acid and salicylic acid on mandarin seedlings are still poorly understood, despite the fact that they are employed in many crops to promote growth. Therefore, identifying the role of these substances in enhancing mandarin seedling performance is essential to address the problem of slow growth and improve nursery productivity, which leads to the use of methods and means to accelerate the seedlings to reach the appropriate size, including spraying the shoots with plant growth regulators, which It has an important and significant role in many important physiological activities within the plant and regulating plant growth [9,10], and fertilizing it with fertilizers, especially major and minor sprays, has a role in stimulating growth and reducing the use of fertilizer through the soil. Which may cause a percentage of it to be lost.

Organic acids, including humic acid, considered stimulants that have a direct and influential role in improving the nutritional status of a plant and activating many vital activities in its tissues that increase its growth and development [11 12]. It stimulates enzymes, vitamins, and hormones within plant tissues, increases the activity of plants, has a role in improving cell division and elongation, and increases the permeability of cellular membranes and the efficiency of the photosynthesis process. The use of organic fertilizers, including humic acid, has a positive effect on soil health and reduces

the use of heavy inorganic fertilizers to increase production [13]. Salicylic acid considered one of the compounds of a phenolic nature that plants produce on a large scale. It has an important physiological role in plant growth, flowering, and absorption of ions, as well as its effect on the movement of stomata. It also works to accelerate the formation of chlorophyll and carotene pigments, the process of photosynthesis, and the activity of some enzymes. Important in plants, given the physiological role of salicylic acid in plant growth and development and a number of plant hormones [14]. The application of salicylic acid improved the vegetative and chemical growth indicators of the plant [15]. [16] reported that SA affects IAA production and transport, altering the patterning of Arabidopsis root meristems.

Materials and Methods

The experiment was conducted under a wooden canopy in one of the nurseries located in Al-Najaf Al-Ashraf, Kufa / Al-Issa region during the 2020–2021 growing season. One-year-old seedlings of mandarin (*Citrus reticulata*), grafted onto *Citrus aurantium* rootstock, were selected and unified as much as possible in their vegetative growth. The seedlings were initially planted in a sandy soil mixture inside 5-kg plastic containers. They were later transplanted into 10-kg plastic pots to provide a larger soil volume capable of supporting natural root development and preventing root restriction throughout the experimental period, thereby improving the accuracy and reliability of the results. All horticultural practices, including insect control, hoeing, fertilization, and irrigation, were applied uniformly to all treatments.

A composite soil sample was taken after thoroughly mixing the soil to determine its physical and chemical properties (Table 1). The experiment was arranged as a factorial experiment with two factors, including 27 treatments and a total of 81 seedlings (three seedlings per treatment). The first factor consisted of humic acid at three concentrations: 0, 5, and 10 mL L⁻¹ (H0, H1, H2). The second factor consisted of salicylic acid at concentrations of 0, 50, and 100 mg L⁻¹ (S0, S1, S2). Before each spray application, the plants were irrigated abundantly one day in advance to enhance the efficiency of foliar absorption. On the following morning, foliar spraying was carried out using a 5-liter hand sprayer until complete leaf wetness was achieved. Six spray applications were performed in total, with two-week intervals between each spray, extending from 15 February to 1 July 2021. A wetting/dispersing agent (Tween-20) was added to all spray solutions at a concentration of 0.1 mL L⁻¹ to improve coverage and penetration of the applied substances. At the end of June 2021, all vegetative and chemical characteristics included in the study were measured.

Table (1): Some physical and chemical characteristics of the soil used in the experiment

Analysis type	Analysis value	unit of measure
Clay	2.4	g kg ⁻¹
Sand	97.0	
Silt	7.6	
N	2.76	mg L ⁻¹
P	287	
K	2.33	
Ca	33.23	
Na	15.7	
Fe	0.83	
pH	7.4	-----
EC	2.26	ds.m ⁻¹
Organic material	13.8	g kg ⁻¹
Soil texture	Sandy mixture	-----

Studied traits

1. Height of the seedling was measured from the point of contact with the soil to the end of the plant's growing top(cm).
2. stem diameter (Vernia caliper) was used to measure the primary stem diameter (mm) at the place where the fifth true leaf touched the stem.
3. Number of leaves: The number of fully developed leaves on the plant was determined.
4. Leaf area (cm²): Three fully expanded leaves were selected from each plant in the experimental unit, and their area was determined following the method accredited by [18]. was measured by scanning the leaves and analyzing the images using the ImageJ software.
5. Number of branches (shoot seedlings⁻¹): The quantity of fresh seedling growths was computed.
6. Carbohydrate content in the diet: The acid colorimetric method (PSALM) Modification of phenol sulphuric described by [17] was used to estimate this property.
7. Total chlorophyll content in leaves (mg 100g⁻¹ fresh weight): It was calculated using the methodology. [18].

Statistical analysis

The experiment was arranged as a 3 × 3 factorial within a randomized complete block design (RCBD) with three replications. Data were analyzed using SAS software (version 9.1), and two-way ANOVA was applied to evaluate factor effects and their interaction. Treatment means were compared using the Least Significant Difference (LSD) test at the 0.05 probability level according to [19].

Results and Discussion

Seedling height (cm)

The Table2 shows that foliar application of humic acid at concentrations 10 ml L^{-1} had a significant effect, surpassing the other treatments and producing the greatest seedling height (27.85 cm). In contrast, the control treatment recorded the lowest seedling height (16.75 cm). According to Table (2), foliar application of salicylic acid at 100 mg L^{-1} significantly increased seedling height compared with the control, reaching 25.07 cm. Regarding the relationship between humic acid and the growth regulator Salicylic acid, there was a significant difference between the treatments, as the treatment excelled (10 + 100) ml L^{-1} respectively, by obtaining the highest rate of seedling length, which reached (31.80 cm) compared to the lowest rates (15.38 cm) in the comparison treatment.

Table (2): The effect of spraying with humic acid and salicylic acid and their interaction on seedling height (cm)

Humic acid Mg L^{-1}	Salicylic acid ml L^{-1}			Humic acid average
	0S	S1	S2	
H0	15.38	16.06	18.79	16.75
H1	20.19	22.46	24.6	22.42
H2	24.84	26.90	31.80	27.85
average Salicylic acid	20.14	21.81	25.07	X
L.S.D0.05	Humic acid		Salicylic acid	Interaction S. H
	0.367		0.367	0.634

Stem diameter (mm)

The results in Table (3) demonstrate that foliar application of humic acid at a concentration of 10 ml L^{-1} produced a significant increase in seedling stem diameter, reaching 4.77 mm, whereas the control treatment recorded the lowest value (3.66 mm). Regarding salicylic acid, spraying with the 100 ml L^{-1} concentration significantly reduced the stem diameter to 4.57 mm compared with the control. Regarding humic acid and salicylic acid's interaction, there is a significant difference was observed among the treatments, with the combination of 10 + 100 ml L^{-1} achieving the highest stem diameter (4.89 mm), while the control treatment recorded the lowest value (3.40 mm).

Table (3): The effect of spraying with humic acid and salicylic acid and their interaction on stem diameter (mm)

Humic acid Mg L ⁻¹	Salicylic acid ml L ⁻¹			average Humic acid
	0S	S1	S2	
H0	3.40	3.54	4.03	3.66
H1	4.15	4.35	4.47	4.32
H2	4.64	4.78	4.89	4.77
average Salicylic acid	4.06	4.22	4.57	
L.S.D0.05	Humic acid		Salicylic acid	Interaction S. H
	0.010		0.010	0.020

Number of leaves (leaf seedlings⁻¹)

The results in Table (4) show that humic acid has a major impact on the number of leaves per seedling, with the 10 ml L⁻¹ concentration producing the highest value (36.62 leaf seedlings⁻¹), in contrast to the control treatment's lowest number (23.08 leaf seedlings⁻¹). Salicylic acid at a concentrated of 100 ml L⁻¹ also showed a significant increase in leaf number, reaching (33.84 leaf seedlings⁻¹), compared to (26.47 leaf seedlings⁻¹) in the control. Regarding the interaction between humic acid and salicylic acid, a significant difference was observed among treatments, with the combination of 10 + 100 ml L⁻¹ achieving the highest leaf seedlings⁻¹ (43.47), while the control recorded the lowest value (19.71).

Table (4): The effect of spraying with humic acid and salicylic acid and their interaction on the number of leaves

Humic acid Mg L ⁻¹	Salicylic acid ml L ⁻¹			average Humic acid
	0S	S1	S2	
H0	19.71	23.39	26.12	23.08
H1	27.52	29.79	31.93	29.75
H2	32.17	34.23	43.47	36.62
average Salicylic acid	26.47	29.14	33.84	
L.S.D0.05	Humic acid		Salicylic acid	Interaction S. H
	0.97		0.97	1.69

Leaves area (cm²)

The results shown in Table (5) show that humic acid has a substantial impact on leaf area, with the 10 ml L⁻¹ concentration producing the highest value (10.45 cm²) compared to the lowest value observed in the control treatment (7.48 cm²). Salicylic acid at 100 ml L⁻¹ also had a significant effect, increasing leaf area to 9.70 cm², compared with 8.32 cm² in the control. Regarding Humic acid and salicylic acid interaction, a notable distinction was observed among treatments, with the combination of 10 + 100 ml L⁻¹ achieving the highest leaf area per seedling (11.81 cm²), while the control recorded the lowest value (7.04 cm²).

Table (5): The effect of spraying with humic acid and salicylic acid and their interaction on leaf area (cm²)

Humic acid Mg L ⁻¹	Salicylic acid ml L ⁻¹			average Humic acid
	0S	S1	S2	
H0	7.04	7.32	8.09	7.48
H1	8.53	8.78	9.20	8.84
H2	9.38	10.18	11.81	10.45
average Salicylic acid	8.32	8.76	9.70	X
L.S.D0.05	Humic acid		Salicylic acid	Interaction S. H
	0.145		0.145	0.251

Number of shoots (shoot seedlings⁻¹)

Table (6) shows that humic acid has a major impact on the number of shoots per seedling, with the 10 ml L⁻¹ concentration producing the highest value (2.77 shoot seedlings⁻¹) as opposed to the lowest value recorded in the control treatment (1.43 shoot seedlings⁻¹). Salicylic acid at a concentration of 100 ml L⁻¹ also significantly increased shoot number to (2.39 shoot seedlings⁻¹), compared with (1.83 shoot seedlings⁻¹) in the control. Regarding humic acid and salicylic acid interaction, a notable distinction was observed among treatments, with the combination of 10 + 100 ml L⁻¹ achieving the highest number of shoot seedlings⁻¹ (3.19), while the control treatment recorded the lowest value (1.16) shoot seedlings⁻¹.

Table (6): The effect of spraying with humic acid and salicylic acid and their interaction on the number of seedling shoots (shoot seedlings⁻¹)

Humic acid Mg L ⁻¹	Salicylic acid ml L ⁻¹			average Humic acid
	0S	S1	S2	
H0	1.16	1.4	1.68	1.43
H1	1.88	2.10	2.30	2.09
H2	2.45	2.67	3.19	2.77
average Salicylic acid	1.83	2.07	2.39	
L.S.D0.05	Humic acid		Salicylic acid	Interaction S. H
	0.047		0.047	0.081

Leaf total chlorophyll content (mg 100 g⁻¹ fresh weight)

Table (7) indicates that humic acid has a significant effect on the total chlorophyll content of leaves, with the 10 ml L⁻¹ concentration producing the highest value (116.77 mg 100 g⁻¹ fresh weight), While the control treatment gave the lowest value (69.89 mg 100 g⁻¹ fresh weight). Salicylic acid at 100 ml L⁻¹ also significantly increased total chlorophyll content to 99.17 mg 100 g⁻¹ fresh weight, compared with 83.89 mg 100 g⁻¹ fresh weight in the control. Regarding Salicylic acid and humic acid interaction, a significant difference was observed among treatments, with the combination of 10 + 100 ml L⁻¹ achieving the highest leaf total chlorophyll content (126.52 mg 100 g⁻¹ fresh weight), while the control recorded the lowest value (63.85 mg 100 g⁻¹ fresh weight).

Table (7): The effect of spraying with humic acid and salicylic acid and their interaction on the total chlorophyll content of leaves (mg 100 g⁻¹ fresh weight)

Humic acid Mg L ⁻¹	Salicylic acid ml L ⁻¹			average Humic acid
	S0	S1	S2	
H0	63.85	9.46	76.35	69.89
H1	81.55	87.43	94.63	87.87
H2	104.78	119.00	126.52	116.77
average Salicylic acid	83.89	91.96	99.17	
L.S.D0.05	Humic acid		Salicylic acid	Interaction S. H
	1.63		1.63	2.82

Leaf total carbohydrate content (%)

Table (8) shows that humic acid contributed significantly to on the total carbohydrate content in leaves, with the 10 ml L⁻¹ concentration producing the highest value (11.73%) in compared to the control treatment's lowest value (7.36%). Salicylic acid at a concentration of 100 ml L⁻¹ also significantly increased leaf total carbohydrates to 10.05%, compared with 8.75% in the control. Regarding humic acid and salicylic acid interaction, a notable distinction was observed among treatments, with the combination of 10 + 100 ml L⁻¹ achieving the highest leaf total carbohydrate content (12.54%), while the control recorded the lowest value (7.26%).

Table (8): The effect of spraying with humic acid and salicylic acid and their interaction on the percentage of total carbohydrates in the containers

Humic acid Mg L ⁻¹	Salicylic acid ml L ⁻¹			average Humic acid
	0S	S1	S2	
H0	7.26	7.35	7.46	7.36
H1	8.08	9.21	10.16	9.15
H2	10.92	11.73	12.54	11.73
average Salicylic acid	8.75	9.43	10.05	X
L.S.D0.05	Humic acid		Salicylic acid	Interaction S. H
	0.15		0.15	0.30

From Tables (2, 3, 4, 5, 6, 7, 8), The results indicated a significant increase in all the studied traits, including stem height, stem diameter, number of leaves, number of branches, leaf area, leaf chlorophyll content, and leaf total carbohydrate percentage, when seedlings were treated with salicylic acid (S2), which showed superiority for all the measured characteristics. The reason for the increase can be attributed to the fact that spraying with salicylic acid led to a growth-stimulating effect in terms of encouraging physiological processes and their impact on the plant's ability to absorb water, nutrients, and mineral elements, which leads to improved plant growth [20]. The observed increase in the aforementioned traits may be attributed to the role of salicylic acid as a plant hormone, which stimulates enzymes involved in photosynthesis and enhances the efficiency of these processes, leading to greater accumulation of synthesized nutrients within the plant, which resulted in an increase in the aforementioned traits [21]. The increase in seedling height can be mechanistically attributed to enhanced cell division and elongation, stimulated by both humic acid and salicylic acid. Salicylic acid further contributed to height increase through its role in modulating auxin

distribution, which regulates apical dominance and stem elongation. Moreover, SA improved chloroplast function and thylakoid integrity, leading to more efficient light capture and photosynthesis. This enhanced chlorophyll content and, consequently, carbohydrate accumulation, which provided the energy and structural components required for cell expansion and tissue differentiation. The reason is also attributed to it providing the nutrients that the plant needs, which are involved in building the chlorophyll molecule, nucleic acids, and proteins, and then increasing the seedlings' ability to carry out the process of photosynthesis and thus manufacturing nutrients that have a role in cell division and elongation [22, 23, 24], these results are consistent with those reported by [25], who found that spraying papaya seedlings with salicylic acid and humic acid, particularly at higher concentrations, resulted in the most pronounced improvements in most studied traits. Similarly, [26] reported that foliar application of salicylic acid on tangerine seedlings grafted onto sour orange rootstock enhanced their chemical characteristics.

The data in the same tables (2, 3, 4, 5, 6, 7, 8) also indicate that treatment with humic acid had a significant effect on the studied traits if the treatment (H2) was significantly superior, the nutritional elements present in humic acid and their role in the excellent growth of the plants, particularly the major ones (N, K), and its role in activating the processes of respiration and photosynthesis, as most of these elements are included in the synthesis of amino and nuclear acids, as well as enzymes and proteins that lead to an increase and encouragement of cell division, as well as cell elongation and tissue growth, which leads to the activation of the cambium layer, which leads to increased vegetative and root growth [27, 28]. The reason is also attributed to the effect of humic acid in the formation of a strong and large root system, which increased the efficiency of absorption of water and nutrients and increased the activity of the carbon metabolism process, thus increasing the materials manufactured in the plant such as sugars and starch, and thus increasing the dry materials of the shoot and root system of the seedling [29, 30, 31]. The results were consistent with [32]. Using humic sprays on Citrus unanatifolia Swingle seedlings led to a significant improvement in the tree's size and growth in terms of stem length, number of leaves and branches, leaf area, canopy size, and leaves' content of elements, [33]. When humic acid was applied to mandarin seedlings, the results showed a profound response to the treatments on vegetative and chemical growth parameters, [34]. The effect of foliar spraying with humic acid and seaweed extract on the growth of local apricot seedlings, and [35] When spraying tangerine seedlings with humic acid. The stem's length, diameter, number of leaves, leaf area, and chlorophyll content all significantly increased as a result. The highest seedling height and overall growth performance were achieved by the synergistic impact of applying humic acid and salicylic acid together. Salicylic acid maximized physiological signaling and metabolic activity, while humic acid stimulated a strong root system to

improve water and nutrient intake and maximize vegetative development. Improved nutrient absorption, increased carbohydrate synthesis, and coordinated hormone-mediated growth regulation are all integrated in the observed increases in stem length, leaf number, branch number, leaf area, chlorophyll content, and total carbohydrate, providing a mechanistic explanation for the improved seedling growth [36], The study was consistent with [37], when applying the spray to pomegranate and lemon seedlings; foliar spraying with humic and salicylic acid (and also urea) resulted in a significant increase in vegetative and root growth and the content of elements in the leaves (N, P, K, Fe, Mn, Zn).

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