Effect of nano and organic fertilizers on Chemical traits of local orange seedlings grafted on two different rootstocks

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Abstract

The experiment was conducted in lathhouse of the Plant Production Department at Al-Musaib Technical College during autumn and spring growing seasons of 2023 and 2024. To study the effect of nano-fertilizers and seaweed extract on the growth of local orange seedlings grafted on two different rootstocks (sour orange and Volkameriana) at the age of (one year) .The experiment included three factors, the first factor is the grafted varieties, the variety grafted on sour orange and Volkameriana and symbolized by the symbol (A1 and A2), while the second factor is the use of seaweed extract, which was added to the soil after planting at three levels (0, 10, 20 g. seedling-1) and symbolized by the symbol (B1, B2, B3). The third factor is spraying with nano-fertilizer (NPK). And at three levels (0, 1.5, 2 g/L) and symbolized by the symbol (C3, C2, C1). A factorial experiment was conducted according to the Randomized Complete Blocks Design (RCBD), with three replicates. The results were analyzed using the Genstat program and compared with the least significant difference (L.S.D) test at a probability level of 0.05. The results were summarized as follows: The results showed that the rootstock had a significant effect on chemical traits of orange seedlings, as the rootstock of sour orange (A1) was significantly excelled on the rootstock of volcameriana (A2) and gave the highest rate for traits of percentage of nitrogen, Phosphorus and potassium in leaves (1.85, 0.52, 2.63%) respectively, leaf content of iron 34.06 mg.kg-1 dry weight), leaf content of catalase and peroxidase (0.18 and 2.87 IU.g-1 fresh weight) respectively. 2- The treatment of adding seaweed extract at a concentration of 20 g. pot-1 (B3) was significantly excelled and gave the highest rate of percentage of nitrogen and phosphorus Potassium in the leaves (2.32, 0.55, 2.76%) respectively, the content of iron in the leaves 36.96 mg.kg-1 dry weight), the content of catalase and peroxidase in the leaves (0.22 and 3.17 IU.g-1 fresh weight) respectively. The treatment of spraying nano NPK at a concentration of 2 mg.L-1 (C3) the highest rate for the studied traits. The triple interaction treatment consisting of (stem of the sour orange seed and seaweed extract at a concentration of 20 g. Pot-1 and spraying with nano fertilizer NPK at a concentration of 2 g. L-1) significantly excelled on the rest of the interaction treatments and recorded the highest values for the traits of percentage of nitrogen, phosphorus and potassium in leaves (2.96, 0.95, 2.90%) respectively, leaf iron content 47.26 mg.kg-1 dry weight, leaf catalase and peroxidase content (0.35 and 4.13 IU.g-1 fresh weight) respectively

Keyword: nano-fertilizers , seaweed extract, orange, peroxidase, catalase

Introduction

Citrus fruits are evergreen fruit plants that belong to the Rutaceae family and include many genera, the most important of which is the Citrus genus, which includes four groups: the lemon group, the acid group, the orange

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group, and the hybrid group [1]. The orange tree is an evergreen tree, but its leaves are renewed, producing annually a number of leaves exceeding those that fell in the same season. Its flowers are white in color and have a pleasant smell. Citrus fruits are of a special type, berries (Hesperidium). [2,3] Commercial varieties of the genus Citrus are propagated on appropriate rootstocks. Given that the tree depends in its basic structure on two main parts, which are the graft represented by the green group and the rootstock such as the seedling orange rootstock and the volcameriana which represents the root group and is part of the stem and the physiological relationship that links them, it is known that the rootstock has an effect on several characteristics of the grafted variety, including the shape, size and nature of tree growth, the beginning of pregnancy and production, and tolerance to environmental factors such as soil and climate, as well as widespread diseases. Therefore, it is considered one of the most important requirements for its use as a rootstock. The slow growth of different citrus rootstocks and the long period of time for them to reach the stage suitable for grafting are among the main problems that lead to an increase in their production costs, which calls for the use of means to accelerate the seedling's arrival to the appropriate size for grafting, including the use of nano and biofertilizers [4] Nanotechnology Among the modern techniques in agricultural production, and since most of the added fertilizer elements deteriorate due to many factors, including washing, adsorption and sedimentation, it is necessary to reduce the loss of nutrients in fertilization, by adopting new applications and with the help of nanotechnology and nanomaterials, nanofertilizers or nanonutrient encapsulations have emerged that have effective properties to accelerate seedling growth and release nutrients on demand, and control the release of nutrients that regulate plant growth and enhance its targeted activity [5]. Foliar (non-root) nutrition is one of the best fertilization technologies for nutrients in the nano form, as it helps in high utilization of nutrients and reducing environmental pollution. [6]. As for seaweed extract, it is considered an essential element in achieving healthy and sustainable growth for orange trees, as it provides the necessary nutrients such as nitrogen, phosphorus and potassium in a sustainable manner. These fertilizers contribute to improving soil properties and enhancing soil structure to facilitate the absorption of nutrients and water by the roots, as the modern scientific trend has begun to use natural compounds and move away from chemical compounds [7]. The research aims to investigate the Effect of nano and organic fertilizers on Chemical traits of local orange seedlings grafted on two different rootstocks

Materials and Methods

The experiment was conducted in lathhouse of the Plant Production Department at the Technical College, Al-Musayyab during autumn and spring growing seasons of 2023 and 2024. To study the effect of nano-fertilizers and seaweed extract on the growth of local orange seedlings grafted on two different rootstocks (sour orange and Volkameriana) at the age of (one year) which were obtained from the certified citrus seedling production nursery belonging to the Iraqi Ministry of Agriculture / General Directorate of Horticulture and Forestry in Al-Hindiyah District / Karbala Governorate. Local orange seedlings grafted on two different rootstocks (sour orange) and sour lemon (Volkameriana) at the age of (one year) were selected. Homogeneous in growth as

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much as possible, planted in black plastic bags, these seedlings were transferred to plastic pot with a capacity of (8 kg) after filling them with river mix with peat moss at a ratio of (1:3), and service operations were carried out on them equally for all seedlings in the shade covered with green agricultural net (Saran), the spraying process was carried out in the morning until the seedlings were completely wet, and the control treatment was sprayed with water only, after the field was irrigated one day before the spraying process to increase the efficiency of the plants in absorbing the sprayed material, as humidity plays a role in the process of swelling the guard cells and opening the stomata, in addition to the fact that irrigation before spraying works to reduce the concentration of solutes in the leaf cells, which increases the penetration of the spray solution ions into the leaf [8.[

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

units	**Values**	Properties
g kg^{-1} soil	612	Sand
g kg^{-1} soil	165	Clay
g kg^{-1} soil	223	Silt
-	Sandy loam	Soil texture
-	7.08	pH
DS. m^{-1}	2.50	Electrical conductivity
g. kg^{-1}	7.22	Organic matter M.O
mg. kg^{-1} soil	17.23	Nitrogen
mg. kg^{-1} soil	5.84	Phosphorus
mg. kg^{-1} soil	122.18	Potassium

Study factors:

The factors were as follows:

The first factor (A):- Where the rootstock includes two

A1 = the rootstock of the sour orange

A2 = the rootstock of the Volkameriana

The second factor (B)): Using seaweed extract (soil helpmate produced by the American company Eco safe consisting of (NPK at a rate of 30%, seaweed 10%, trace elements 1%, organic materials 45%, moisture 10%) was added to the soil after planting and at three levels:

- 1Control treatment (0) without addition symbolized by the symbol B1
- 2Adding (10) grams/seedling symbolized by the symbol B2
- 3Adding (20) grams/seedling symbolized by the symbol B3

The third factor is spraying with nano fertilizer (NPK) produced by the Iranian company Khazra, the proportions of nano elements (NPK) in it (20-20-20 %) respectively and at three levels (0, 1.5, 2 g. L-1) (C3, C2,, C1) and the date of the first spray was 10/7/2023.

-1Control treatment (0) without adding water spray only, symbolized by the symbol C1

-2Spraying with a concentration of (1.5 g. L-1) and symbolized by the symbol C2

-3Spraying with a concentration of (2 g. L-1) and symbolized by the symbol C2

Experimental design and analysis:

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A factorial experiment (2*3*3) was implemented according to the Randomized Complete Blocks Design (RCBD), on (270) seedlings with three replicates, as each replicate contains (18) treatments with (5) seedlings for each experimental unit, and the results were analyzed using the Genstat program and compared with the least significant difference (L.S.D) test at a probability level of 0.05 and according to the method mentioned In [9.[

Periods of spraying and ground fertilization:
Orange seedlings grafted on sour orange and Volkameriana were sprayed and ground fertilized at a rate of 3 sprays every (15) days between one spray and another for the autumn season. In November 10/8/2023, 10/23/2023, 11/7/2023 and the spring season on (3/1/2024, 3/16/2024, 4/1/2024(

Table (2) Components of seaweed extract

% Percentage	Nutritional elements	No.
%4	**N**	1
%4	**P2O5**	2
% 4	**k2O**	3
32**ppm**	**Mg**	4
30**ppm**	**Fe**	5
31**ppm**	**Mn**	6
17.5**ppm**	**Zn**	7
12.6**ppm**	**Cu**	8

Studied traits:
Percentage of nitrogen, phosphorus and potassium in the leaves:
Leaf samples were taken from each seedling and each replicate, then washed with distilled water to get rid of dust and impurities attached to them, and placed in perforated paper bags, then dried in an electric oven at a temperature of 70 degrees Celsius for 48 hours until the dry weight was fixed [4]. After that, the samples were ground and 0.5 g of the powder of the ground sample (dry leaves) was taken using an electric grinder and digested with concentrated sulfuric acid and 1 ml of concentrated perchloric acid [14]and the following elements were estimated:

*Nitrogen:(%)
Nitrogen was estimated using a microcalculator [8[
*Phosphorus (%): Phosphorus was estimated using ammonium molybdate and measured using a spectrophotometer at a wavelength of (882nm) according to the method of [17]
*Potassium:(%)
Potassium was determined by Flame photometer according to the method followed by [13[

Catalase activity (absorption unit per g-1 fresh weight(
It was measured according to the method of [1] by taking 1 g of green leaf samples and

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adding 1 ml of potassium phosphate buffer solution (K-Buffer 20, (pH 7 mM with 0.3 g of PVP) Polyvinyl pyrolidone) and crushing it in a pre-cooled ceramic mortar and placing it on ice chips to provide cool conditions that prevent enzyme breakdown, then the extract was filtered with a piece of gauze and the filtrate was centrifuged at 10000 rpm for ten minutes at 4°C. Then 20 microliters of the filtrate (enzymatic extract) were taken and 1 ml of 30% hydrogen peroxide solution was added to it, then incubated at 25°C for one minute. Then, spectrophotometer readings were taken at a wavelength of 240 nm. The readings were repeated after one minute from the first reading time for each sample. It was noted that there was a decrease in absorbance over time. The device was zeroed using the comparison solution (Blank) prepared from the same materials, replacing the basic material with potassium phosphate buffer solution. The enzyme activity was calculated using the following equation: Enzyme activity (absorbance unit/gram fresh weight) = (difference in absorbance/time) × volume of enzyme extract/0.001

Peroxidase enzyme activity (absorption unit g-1 fresh weight:(

The method described in [16] was followed, as the enzyme sample was prepared by taking 100 mg of green leaf samples and crushing them in a ceramic mortar with a quantity of 0.1 M potassium phosphate (K-Buffer) solution, which was prepared as follows:

-1Dissolve 1.3609 g of $KH_2 PO_4$ (molecular weight 136.09 g/mol) in 100 ml of distilled water (acidic solution.(

Dissolve 1.7418 g of K_2HPO_4 (molecular weight 174.18 g/mol) in 100 ml of distilled water (basic solution.(

I took a sufficient quantity of KH_2PO_4 for measurements and adjusted its acidity (pH) to 6.7 by adding K_2HPO_4. Then it was transferred to a 25 ml standard flask and the volume was completed with phosphate buffer solution. After 15 minutes, the sample was centrifuged at 4000 rpm for 10 minutes. Then 0.12 ml of the filtrate was taken and placed in a measuring tube. 1.1 ml of distilled water, 0.8 ml of phosphate buffer solution, 0.5 ml of hydrogen peroxide (H_2O_2) 0.15% and 0.5 ml of Gauiacol dye (prepared on the same day of the estimation by dissolving 182 mg of Gauiacol in 25 ml of distilled water) were added. Then the intensity of the color resulting from the oxidation of the reaction material was read directly using a spectrophotometer with a wavelength of 470 nm after the device was zeroed using the comparison solution (Blank) prepared by adding distilled water instead of hydrogen peroxide. The enzyme activity was calculated using the following equation:

Enzyme activity (absorption unit/gram fresh weight) = reading of the device/(sample weight/extraction volume) volume taken for reading

Results and discussion

Percentage of nitrogen in the leaves(%)

The results in Table (3) showed that the rootstock had a significant effect on the percentage of nitrogen in the leaves (%), as sour orange rootstock (A1) was significantly excelled and gave the highest percentage of nitrogen in the leaves, reaching 1.85%, while the volcamerian rootstock (A2) gave the percentage of nitrogen in the leaves, 1.11%.

The results also showed that seaweed extract had a significant effect on the percentage of nitrogen in the leaves, as the concentration of 20 g.pot-1 (B3) was significantly excelled and gave the highest rate of nitrogen percentage in the leaves, reaching 2.32%, followed by the concentration of 10 g.pot-1 (B2) and recorded a percentage of nitrogen in the leaves of

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1.90%, while the treatment without addition (B1) gave the lowest rate of nitrogen percentage in the leaves, reaching 1.60%, while the treatment of spraying nano fertilizer with a concentration of 2 mg. L-1 (3C) was superior and recorded the highest rate of nitrogen percentage in the leaves, reaching 1.95%, followed by the spraying treatment with a concentration of 1.5 mg. L-1 (2C) and gave a rate of nitrogen percentage in the leaves of 1.47%, while the treatment without spraying (1C) recorded the lowest rate of nitrogen percentage in the leaves It reached 1.30%. The results also showed that the bi-interaction had a significant effect on the percentage of nitrogen in the leaves. The interaction treatment between the seeded sour orange and seaweed extract at a concentration of 20 g.pot-1 was excelled and recorded the highest rate of 2.07%, while the treatment of the rootstock of the volcameriana and the treatment without adding seaweed extract recorded the lowest rate of nitrogen percentage in the leaves, reaching 1.09%. While the interaction treatment between the rootstock of the sour orange (A1) and spraying nano-fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of 2.16%, while the treatment of the rootstock of the volcameriana ((A2 and without spraying nano-fertilizer (1C) gave the lowest percentage of nitrogen in the leaves, reaching 1.11%. The interaction treatment between adding seaweed extract at a concentration of 20 g.pot-1 (B3) and spraying nano-fertilizer at a concentration of 2 mg. L-1 was also recorded. (3C) gave the highest rate of nitrogen percentage in the leaves, reaching 2.65%, while the treatment without adding seaweed extract (B1) and without spraying nano-fertilizer (1C) recorded the lowest rate of nitrogen percentage in the leaves, reaching 1.12%.The results of Table (3) also showed that the triple interaction between the experimental factors had a significant effect on the percentage of nitrogen in the leaves. The interaction factor between the rootstock of the orange seed and seaweed extract at a concentration of 20 g.pot-1 and spraying nano-fertilizer at a concentration of 2 mg. L-1 was significantly excelled and gave a rate of 2.96%, while the interaction treatment between the rootstock of the volcameriana and the addition of seaweed extract (B1) and without spraying nano-fertilizer (1C) gave the lowest rate of 1.09%.

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Table (3) Effect of the rootstock, seaweed extract , spraying nano-fertilizer and the interaction between them on the percentage of nitrogen in the leaves (%) of the orange plant

A x B	Nano Fertilizer Spraying) **C**(			seaweed extract) **B**(	rootstock)**A**(
	Spray 2 mg.L-1 **3)**C**(**	Spray 1.5 mg.L-1**2)** C**(**	Without spraying **1)C**(		
1.26	1.57	1.10	1.10	Without adding**(B1**)	sour orange **(A1)**
1.38	1.89	1.15	1.11	**10**g.pot-1) **B2(**	
2.07	2.96	1.29	1.96	**20**g.pot-1) **B3**(	
1.09	1.12	1.07	1.09	Without adding**(B1**)	Volkameriana) **(A2**
1.16	1.24	1.12	1.11	**10**g.pot-1) **B2(**	
1.20	1.31	1.17	1.12	**20**g.pot-1) **B3**(	
0.08	0.14			**L.S.D 0.05**	
rootstock)**A** (	Interaction between rootstock and Nano Fertilizer Spraying				
1.85	2.86	1.56	1.12	sour orange) **A1**(	
1.11	1.99	1.41	1.11	Volkameriana**) (A2**	
0.05	0.08			**L.S.D 0.05**	
seaweed extract)**B**(	Interaction between seaweed extract and Nano Fertilizer Spraying				
1.60	1.80	1.89	1.12	Without adding**(B1**)	
1.90	2.06	2.14	1.50	**10**g.pot-1) **B2(**	
2.32	2.65	2.32	1.98	**20**g.pot-1) **B3**(	
0.06	0.10			**L.S.D 0.05**	
	1.95	1.47	1.30	Nano Fertilizer Spraying) **C**(	
	0.06			**L.S.D 0.05**	

Percentage of phosphorus in the leaves(%)

The results of Table (4) showed that the rootstock had a significant effect on the percentage of phosphorus in the leaves (%). The rootstock of the orange seed (A1) was significantly excelled. Morally, it gave the highest percentage of phosphorus in the leaves, reaching 0.52%, while the Volcameriana rootstock (A2) gave the percentage of phosphorus in the leaves, 0.36%. The results also showed that seaweed

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extract had a significant effect on the percentage of phosphorus in the leaves, as the concentration of 20 g.pot-1 (B3) was significantly excelled and gave the highest rate of phosphorus percentage in the leaves, reaching 0.55%, followed by the concentration of 10 g.pot-1 (B2) and recorded a percentage of phosphorus in the leaves of 0.45%, while the treatment without addition (B1) gave the lowest rate of phosphorus percentage in the leaves, reaching 0.38%, while the treatment of spraying nano fertilizer with a concentration of 2 mg. L-1 (3C) was superior and recorded the highest rate of phosphorus percentage in the leaves, reaching 0.60%, followed by the spraying treatment with a concentration of 1.5 mg. L-1 (2C) and gave a rate of phosphorus percentage in the leaves of 0.46%, while the treatment without spraying (1C) recorded the lowest rate of phosphorus percentage in the leaves. It reached 0.38%. The results also showed that the bi-interaction had a significant effect on the percentage of phosphorus in the leaves. The interaction treatment between the seeded sour orange and seaweed extract at a concentration of 20 g.pot-was excelled and recorded the highest rate of 0.73%, while the treatment of the seeded sour orange rootstock and the treatment without adding seaweed extract gave the lowest rate of phosphorus percentage in the leaves, reaching 0.34%. While the interaction treatment between the seeded sour orange rootstock (A1) and spraying nano-fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of 0.64%, while the treatment of the rootstock of volcameriana ((A2 and without spraying nano-fertilizer (1C) gave the lowest percentage of phosphorus in the leaves, reaching 0.21%. The interaction treatment between adding seaweed extract at a concentration of 20 g.pot-1 (B3) and spraying nano-fertilizer at a concentration of 2 mg. L-1 was also recorded. (3C) and gave the highest rate of phosphorus percentage in the leaves, reaching 0.62%, while the treatment without adding seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of phosphorus percentage in the leaves, reaching 0.29%. The results of Table (4) also showed that the triple interaction between the experimental factors had a significant effect on the percentage of phosphorus in the leaves, as the interaction factor between the rootstock of the sour orange seed and seaweed extract at a concentration of 20 g.pot-1 and spraying nano fertilizer at a concentration of 2 mg. L-1 was significantly excelled and gave a rate of 0.95%, while the interaction treatment between the rootstock of the sour orange seed and adding seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of 0.23%.

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Table (4) Effect of the rootstock, seaweed extract , spraying nano fertilizer and their interaction on the percentage of phosphorus in the leaves (% Orange plant

A x B	Nano Fertilizer Spraying) **C**(			seaweed extract) **B**(	rootstock)**A**(
	Spray 2 mg.L-1 **3)**C**(**	Spray 1.5 mg.L-1**2)** C**(**	Without spraying **1)C**(		
0.34	0.43	0.36	0.23	Without adding**(B1**)	sour orange **(A1)**
0.58	0.73	0.54	0.48	**10**g.pot-1) **B2(**	
0.73	0.95	0.66	0.58	**20**g.pot-1) **B3**(	
0.37	0.4	0.39	0.32	Without adding**(B1**)	Volkameriana) **(A2**
0.38	0.43	0.37	0.34	**10**g.pot-1) **B2(**	
0.61	0.79	0.55	0.49	**20**g.pot-1) **B3**(	
0.03	0.06			**L.S.D 0.05**	
rootstock)**A** (	Interaction between rootstock and Nano Fertilizer Spraying				
0.52	0.64	0.50	0.42	sour orange) **A1**(	
0.36	0.51	0.36	0.21	Volkameriana**)** **(A2**	
0.01	0.03			**L.S.D 0.05**	
seaweed extract)**B**(	Interaction between seaweed extract and Nano Fertilizer Spraying				
0.38	0.47	0.38	0.29	Without adding**(B1**)	
0.45	0.58	0.46	0.31	**10**g.pot-1) **B2(**	
0.55	0.62	0.53	0.49	**20**g.pot-1) **B3**(	
0.02	0.035			**L.S.D 0.05**	
	0.60	0.46	0.38	Nano Fertilizer Spraying) **C**(	
	0.02			**L.S.D 0.05**	

Percentage of potassium in leaves(%)

The results in Table (5) showed that the rootstock had a significant effect on the percentage of potassium in leaves (%). The seeded sour orange rootstock (A1) was significantly excelled and gave the highest percentage of potassium in leaves, reaching

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2.63%, while the volcamerian rootstock (A2) gave the percentage of potassium in leaves, 2.20%.

The results also showed that seaweed extract had a significant effect on the percentage of potassium in the leaves, as the concentration of 20 g.pot-1 (B3) was significantly excelled and gave the highest rate of potassium percentage in the leaves, reaching 2.76%, followed by the concentration of 10 g.pot-1 (B2) and recorded a percentage of potassium in the leaves of 2.44%, while the treatment without addition (B1) gave the lowest rate of potassium percentage in the leaves, reaching 2.31%, while the treatment of spraying nano fertilizer with a concentration of 2 mg. L-1 (3C) was superior and recorded the highest rate of potassium percentage in the leaves, reaching 2.62%, followed by the spraying treatment with a concentration of 1.5 mg. L-1 (2C) and gave a rate of potassium percentage in the leaves of 2.51%, while the treatment without spraying (1C) recorded the lowest rate of potassium percentage in the leaves It reached 2.22%. The results also showed that the bilateral interaction had a significant effect on the percentage of potassium in the leaves. The interaction treatment between the seeded sour orange and seaweed extract at a concentration of 20 g.pot-was excelled and recorded the highest rate of 2.74%, while the treatment of the rootstock of the volcameriana and the treatment without adding seaweed extract gave the lowest rate of potassium percentage in the leaves, reaching 1.95%. While the interaction treatment between the rootstock of the sour orange (A1) and spraying nano-fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of 2.85%, while the treatment of the rootstock of the volcameriana ((A2 and without spraying nano-fertilizer (1C) gave the lowest percentage of potassium in the leaves, reaching 1.90%. The interaction treatment between adding seaweed extract at a concentration of 20 g.pot-1 (B3) and spraying nano-fertilizer at a concentration of 2 mg. L-1 was also recorded. (3C) and gave the highest rate of potassium percentage in the leaves, reaching 2.87%, while the treatment without adding seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of potassium percentage in the leaves, reaching 2.17%.The results of Table (5) also showed that the triple interaction between the experimental factors had a significant effect on the percentage of potassium in the leaves, as the interaction factor between the rootstock of the sour orange and seaweed extract at a concentration of 20 g.pot-1 and spraying nano fertilizer at a concentration of 2 mg. L-1 was significantly excelled and gave a rate of 2.90%, while the interaction treatment between the rootstock of the volcameriana and adding seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of 1.87%.

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Table (5) Effect of the rootstock, seaweed extract , spraying nano fertilizer and the interaction between them on the percentage of potassium in the leaves (% Orange plant

A x B	Nano Fertilizer Spraying) **C**(Spray 2 mg.L-1 **3**)C**(**	Spray 1.5 mg.L-1**2)** C**(**	Without spraying **1)**C(	seaweed extract) **B**(	rootstock)**A**(
2.26	2.43	2.44	1.90	Without adding**(B1**)	sour orange **(A1)**
2.48	2.76	2.50	2.19	**10**g.pot-1) **B2(**	
2.74	2.90	2.82	2.50	**20**g.pot-1) **B3**(	
1.95	1.67	2.32	1.87	Without adding**(B1**)	Volkameriana) **(A2**
2.50	2.84	2.56	2.10	**10**g.pot-1) **B2(**	
2.66	2.87	2.67	2.45	**20**g.pot-1) **B3**(	
0.68	1.25			**L.S.D 0.05**	
rootstock)**A** (	Interaction between rootstock and Nano Fertilizer Spraying				
2.63	2.85	2.66	2.38	sour orange) **A1**(	
2.20	2.57	2.12	1.90	Volkameriana**) (A2**	
0.23	0.68			**L.S.D 0.05**	
seaweed extract)**B**(	Interaction between seaweed extract and Nano Fertilizer Spraying				
2.31	2.47	2.29	2.17	Without adding**(B1**)	
2.44	2.56	2.47	2.30	**10**g.pot-1) **B2(**	
2.76	2.87	2.79	2.63	**20**g.pot-1) **B3**(	
0.45	0.72			**L.S.D 0.05**	
	2.62	2.51	2.22	Nano Fertilizer Spraying) **C**(	
	0.45			**L.S.D 0.05**	

Iron content of leaves (mg kg-1 dry weight(
The results in Table (6) showed that the rootstock had a significant effect on the iron content of leaves (IU.g-1 fresh weight). The seed orange rootstock (A1) was significantly excelled and gave the highest leaf iron content of 34.06 mg kg-1 dry weight, while the volcamerian rootstock (A2) gave the leaf iron content of 32.36 mg kg-1 dry weight. The results also showed that seaweed extract had a

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significant effect on the iron content of leaves, as the concentration of 20 g.pot-1 (B3) was significantly excelled and gave the highest rate of iron content of leaves, reaching 36.96 mg kg-1 dry weight, followed by the concentration of 10 g.pot-1 (B2) and recorded the iron content of leaves, reaching 34.11 mg kg-1 dry weight, while the treatment without addition (B1) gave the lowest rate of iron content of leaves, reaching 30.23 mg kg-1 dry weight, while the treatment of spraying nano fertilizer with a concentration of 2 mg. L-1 (3C) was superior and recorded the highest rate of iron content of leaves, reaching 37.45 mg kg-1 dry weight, followed by the spraying treatment with a concentration of 1.5 mg. L-1 (2C) and gave an average of iron content of leaves, reaching 33.67 mg kg-1 Dry weight, while the treatment without spraying (1C) recorded the lowest rate of leaf iron content of 30.51 mg kg-1 dry weight. The results also showed that the bi-interaction had a significant effect on leaf iron content, as the interaction treatment between the rootstock of the sour orange and seaweed extract at a concentration of 20 g.pot-1 outperformed and recorded the highest rate of 42.06 mg kg-1 dry weight, while the treatment of the rootstock of the volcameriana and the treatment without adding seaweed extract recorded the lowest rate of leaf iron content of 27.39 g. 100 mg kg-1 dry weight, while the interaction treatment between the rootstock of the sour orange (A1) and spraying nano-fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of 38.46 mg kg-1 dry weight, while the treatment of the rootstock of the volcameriana ((A2 and without spraying nano-fertilizer (1C) gave The lowest leaf iron content was 29.06 mg kg-1 dry weight. The interaction treatment between adding seaweed extract at a concentration of 20 g.pot-1 (B3) and spraying nano fertilizer at a concentration of 2 mg. L-1 (3C) gave the highest rate of leaf iron content of 38.41 mg kg-1 dry weight, while the treatment without adding seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of leaf iron content of 28.56 mg kg-1 dry weight. The results of Table (6) also showed that the triple interaction between the experimental factors had a significant effect on the leaf iron content. The interaction factor between the rootstock of the orange seed and seaweed extract at a concentration of 20 g.pot-1 and spraying nano fertilizer at a concentration of 2 mg. L-1 was significantly excelled and gave a rate of 47.26 mg kg-1 Dry weight While the interaction treatment between the rootstock of Volcameriana and the addition of seaweed extract (B1) and without spraying nano-fertilizer (1C) gave the lowest rate of leaf iron content of 25.36 mg kg-1 dry weight

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Table (6) Effect of rootstock, seaweed extract , spraying nano-fertilizer and their interaction on the iron content in leaves (mg kg-1 dry weight) of orange plant

A x B	Nano Fertilizer Spraying) C(			seaweed extract) B(	rootstock)A(
	Spray 2 mg.L-1 3)C(	Spray 1.5 mg.L-12) C(	Without spraying 1)C(		
29.79	34.36	28.76	26.26	Without adding(B1)	sour orange (A1)
34.36	38.86	32.56	31.66	10g.pot-1) B2(	
42.06	47.26	43.06	35.86	20g.pot-1) B3(	
27.39	29.96	26.86	25.36	Without adding(B1)	Volkameriana) (A2
31.46	35.56	31.36	27.46	10g.pot-1) B2(	
39.86	45.46	39.76	34.36	20g.pot-1) B3(	
0.27	0.469			**L.S.D 0.05**	
rootstock)**A**(	Interaction between rootstock and Nano Fertilizer Spraying				
34.06	38.46	32.46	31.26	sour orange) **A1**(	
32.36	35.36	32.66	29.06	Volkameriana**) (A2**	
0.16	0.27			**L.S.D 0.05**	
seaweed extract)**B**(	Interaction between seaweed extract and Nano Fertilizer Spraying				
30.23	31.21	30.91	28.56	Without adding(**B1**)	
34.11	37.06	35.26	30.01	**10**g.pot-1) **B2(**	
36.96	38.41	36.76	35.71	**20**g.pot-1) **B3**(	
0.19	0.33			**L.S.D 0.05**	
	37.45	33.67	30.51	Nano Fertilizer Spraying) **C**(	
	0.19			**L.S.D 0.05**	

Catalase content of leaves (IU. gm-1 fresh weight(

The results in Table (7) showed that the rootstock had a significant effect on the Catalase content of leaves (IU. gm-1 fresh weight). The seed orange rootstock (A1) was significantly excelled and gave the highest Catalase content of leaves, reaching 0.18 IU. gm-1 fresh weight, while the Volcamerian rootstock (A2) gave the Catalase content of leaves, reaching 0.12 IU. gm-1 fresh weight. The results also showed that seaweed extract

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had a significant effect on the leaf content of Catalase, as the concentration of 20 g.pot-1 (B3) was significantly excelled and gave the highest rate of leaf content of Catalase, reaching 0.22 IU. gm-1 fresh weight, followed by the concentration of 10 g.pot-1 (B2) and recorded the leaf content of Catalase, reaching 0.15 IU. gm-1 fresh weight, while the treatment without addition (B1) gave the lowest rate of leaf content of Catalase, reaching 0.11 IU. gm-1 fresh weight, while the treatment of spraying nano fertilizer with a concentration of 2 mg. L-1 (3C) was superior and recorded the highest rate of leaf content of Catalase, reaching 0.24 IU. gm-1 fresh weight, followed by the spraying treatment with a concentration of 1.5 mg. L-1 (2C) and gave a rate of The leaf content of Catalase was 0.20 IU. g-1 fresh weight, while the treatment without spraying (1C) recorded the lowest rate of Catalase leaf content of 0.14 IU. g-1 fresh weight. The results also showed that the bi-interaction had a significant effect on the leaf content of Catalase, as the interaction treatment between the rootstock of the sour orange and seaweed extract at a concentration of 20 g.pot-1 outperformed and recorded the highest rate of 0.28 IU. g-1 fresh weight, while the treatment of the rootstock of the volcameriana and the treatment without adding seaweed extract recorded the lowest rate of Catalase leaf content of 0.19 IU. g-1 fresh weight, while the interaction treatment between the rootstock of the sour orange (A1) and spraying nano-fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of 0.22 IU. g-1 Fresh weight, while the treatment of Volcameriana rootstock ((A2 and without spraying nano fertilizer (1C) gave the lowest leaf content of Catalase, reaching 0.09, and the treatment of interaction between adding seaweed extract at a concentration of 20 g.pot-1 (B3) and spraying nano fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of leaf content of Catalase, reaching 0.24 IU. gm-1 fresh weight, while the treatment without adding seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of leaf content of Catalase, reaching 0.06 IU. gm-1 fresh weight. The results of Table (7) also showed that the triple interaction between the experimental factors had a significant effect on the leaf content of Catalase, as the interaction factor between the seedling sour orange rootstock and seaweed extract at a concentration of 20 gm. Anvil-1 and spraying nano fertilizer at a concentration of 2 mg. L-1 gave a rate of 0.35 IU. g-1 fresh weight, while the interaction treatment between the rootstock of Volcameriana and the addition of seaweed extract (B1) and without spraying nano fertilizer (1C) gave the lowest rate of leaf content of Catalase, which amounted to 0.14 IU. g-1 fresh weight.

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Table (7) Effect of rootstock, seaweed extract , spraying nano fertilizer and their interaction on leaf content of Catalase (IU. g-1 fresh weight) for orange plant

A x B	Nano Fertilizer Spraying) C(Spray 2 mg.L-1 3)C(	Spray 1.5 mg.L-1 2) C(	Without spraying 1)C(	seaweed extract) B(	rootstock)A(
0.20	0.24	0.19	0.16	Without adding(B1)	sour orange (A1)
0.23	0.28	0.22	0.20	10g.pot-1) B2(	
0.28	0.35	0.27	0.23	20g.pot-1) B3(	
0.19	0.23	0.20	0.14	Without adding(B1)	Volkameriana) (A2
0.21	0.26	0.25	0.13	10g.pot-1) B2(	
0.26	0.31	0.28	0.18	20g.pot-1) B3(	
0.03	0.06			**L.S.D 0.05**	
rootstock)A(	Interaction between rootstock and Nano Fertilizer Spraying				
0.18	0.22	0.18	0.13	sour orange) A1(	
0.12	0.14	0.13	0.09	Volkameriana) (A2	
0.01	0.03			**L.S.D 0.05**	
seaweed extract)B(	Interaction between seaweed extract and Nano Fertilizer Spraying				
0.11	0.17	0.10	0.06	Without adding(B1)	
0.15	0.21	0.15	0.10	10g.pot-1) B2(	
0.22	0.24	0.25	0.17	20g.pot-1) B3(	
0.02	0.04			**L.S.D 0.05**	
	0.24	0.20	0.14	Nano Fertilizer Spraying) C(	
	0.02			**L.S.D 0.05**	

Leaf content of POD (international units. gm-1 fresh weight(

The results in Table (8) showed that the rootstock had a significant effect on the leaf content of POD (international units. gm-1 fresh weight). The seed orange rootstock (A1) was significantly excelled and gave the highest leaf content of POD, reaching 2.87 international units. gm-1 fresh weight, while the volcamerian rootstock (A2) gave the leaf

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content of POD, reaching 2.59 international units. gm-1 fresh weight.

The results also showed that seaweed extract had a significant effect on the leaf POD content, as the concentration of 20 g.pot-1 (B3) was significantly excelled and gave the highest rate of leaf POD content of 3.17 IU. g-1 fresh weight, followed by the concentration of 10 g.pot-1 (B2) and recorded the leaf POD content of 2.72 IU. g-1 fresh weight, while the treatment without addition (B1) gave the lowest rate of leaf POD content of 2.34 IU. g-1 fresh weight, while the treatment of spraying nano fertilizer with a concentration of 2 mg. L-1 (3C) was superior and recorded the highest rate of leaf POD content of 3.02 IU. g-1 fresh weight, followed by the spraying treatment with a concentration of 1.5 mg. L-1 (2C) and gave a rate of leaf POD content of POD reached 2.69 IU. g-1 fresh weight, while the treatment without spraying (1C) recorded the lowest rate of leaf POD content of 2.55 IU. g-1 fresh weight. The results also showed that the bi-interaction had a significant effect on leaf POD content, as the interaction treatment between the rootstock of Volcameriana and seaweed extract at a concentration of 20 g.pot-1 outperformed and recorded the highest rate of 3.39 IU. g-1 fresh weight, while the treatment of the rootstock of the sour orange and the treatment without adding seaweed extract recorded the lowest rate of leaf POD content of 2.25 IU. g-1 fresh weight, while the interaction treatment between the rootstock of the sour orange (A1) and spraying nano-fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of 3.28 IU. g-1 fresh weight, while The treatment of Volcameriana rootstock ((A2 and without spraying nano fertilizer (1C) gave the lowest leaf POD content of 2.27, and the treatment of interaction between adding seaweed extract at a concentration of 20 g.pot-1 (B3) and spraying nano fertilizer at a concentration of 2 mg. L-1 (3C) recorded the highest rate of leaf POD content of 3.40 IU. gm-1 fresh weight, while the treatment without adding seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of leaf POD content of 22.44 IU. gm-1 fresh weight. The results of Table (8) also showed that the triple interaction between the experimental factors had a significant effect on the leaf POD content, as the interaction factor between Volcameriana rootstock and seaweed extract at a concentration of 20 g.pot-1 and spraying nano fertilizer at a concentration of 2 outperformed significantly. mg.L-1 and gave a rate of 4.13 IU.g-1 fresh weight, while the interaction treatment between the rootstock of the atomic orange and the addition of seaweed extract (B1) and without spraying nano fertilizer (1C) recorded the lowest rate of leaf content of POD, which amounted to 1.90 IU.g-1 fresh weight.

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Table (8) Effect of rootstock, seaweed extract , spraying nano fertilizer and their interaction on leaf content of POD (IU.g-1 fresh weight) for orange plant

A x B	Nano Fertilizer Spraying) **C**(			seaweed extract) **B**(	rootstock)**A**(
	Spray 2 mg.L-1 **3)**C**(**	Spray 1.5 mg.L-1**2)** C**(**	Without spraying **1)C**(		
2.25	2.66	2.18	1.9	Without adding**(B1**)	sour orange **(A1)**
2.77	2.8	2.78	2.73	**10**g.pot-1) **B2(**	
3.22	3.55	3.15	2.95	**20**g.pot-1) **B3**(	
2.40	2.55	2.50	2.15	Without adding**(B1**)	Volkameria na) **(A2**
2.61	2.92	2.60	2.32	**10**g.pot-1) **B2(**	
3.39	4.13	3.05	2.98	**20**g.pot-1) **B3**(	
0187	0.185			**L.S.D 0.05**	
rootstock)**A** (	Interaction between rootstock and Nano Fertilizer Spraying				
2.87	3.28	2.75	2.57	sour orange) **A1**(	
2.59	2.96	2.55	2.27	Volkameriana**) (A2**	
0.06	0.107			**L.S.D 0.05**	
seaweed extract)**B**(	Interaction between seaweed extract and Nano Fertilizer Spraying				
2.34	2.38	2.2	2.44	Without adding**(B1**)	
2.72	2.62	2.69	2.84	**10**g.pot-1) **B2(**	
3.17	3.4	3.15	2.95	**20**g.pot-1) **B3**(	
0.76	0.131			**L.S.D 0.05**	
	3.02	2.69	2.55	Nano Fertilizer Spraying) **C**(	
	0.076			**L.S.D 0.05**	

It is clear from Tables (3-8) that the rootstock has a significant effect on the chemical properties, and the explanation of this variation may be due to the variation in physiological changes, which include the absorption of mineral elements, the transfer of nutrients, and the production of growth-promoting substances. [5] noted the difference in rootstocks in their response to the effect of fertilizers on seedlings fertilized with it, and perhaps due to the fact that this fertilizer is a

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source of major elements necessary for plant growth and provides the plant with appropriate amounts of these elements, which provides the plant with its necessary needs, and the reason for the difference and variation of rootstocks among themselves in chemical properties and the variation of rootstocks among themselves, this variation is due to the genetic difference between rootstocks resulting from the variation of genetic factors, which were positively reflected in the physiological processes necessary for vegetative and root growth [12] Thus, increasing the absorption of elements and the formation of enzymes inside the plant and agrees with Ibrahim et al. (2014) that the origins differ from each other according to their genetic characteristics. The results of Tables (3-8) also showed the role of nano-fertilization with NPK on chemical properties, as the treatment of spraying 2 mg.L-1 (3C) significantly outperformed the rest of the other treatments and gave the highest rate of nitrogen, phosphorus and potassium content in the leaves of seedlings. Table (3-5) Treatment with nano-NPK fertilizer may be due to the direct absorption of these elements present in the nano-fertilizer through the leaves, which leads to an increase in its concentration in them. The positive effect of nano-fertilizer in forming a strong green and root group increased the efficiency of the roots' absorption of other nutrients, which increased their concentration inside the plant, in addition to the high entry of nutrient particles in nano-fertilizer requires the withdrawal of the necessary nutrients to complete the photosynthesis process [18] Recent studies confirm that foliar spraying with materials containing major and minor elements leads to an increase in their content within the plant tissue, which explains the increase in the leaf content of elements (Han et al., 2008). This coincides with what [6] mentioned that the elements N, P, and K work to increase the rate of vital processes in which the compounds of these elements participate to form the basic compounds for the process of carbon metabolism and respiration, which increases the readiness of these elements in the leaf. These results agree in terms of increasing the levels of elements in the leaves of the plant when sprayed with nano sources of nitrogen, phosphorus, and potassium with what[11] [17] in figs, and in olives: We note from the results of Tables (3-8) that the reason for the increase in the leaf content of elements may be due to And enzymes when adding seaweed extract to the power of vegetative and root growth of orange seedlings, which positively affects the leaf content of elements, which improved the physiological processes, which was positively reflected in the ability to absorb water and major and minor elements [3]. The reason may be attributed to the fact that adding seaweed extract contributed to increasing the efficiency of absorption of nutrients from the roots and thus increasing their percentage in the leaves, in addition to increasing the ability of the roots to absorb nutrients from the soil when adding organic fertilizers because they increase the effectiveness of the roots and increase their respiration and then produce the energy necessary to absorb some nutrients, and as a result, plant growth and production improve [2]. These elements are also absorbed by plant roots and release their ions easily and move quickly for the plant to benefit from them by participating in physiological processes and providing them to the plant [7]. From the same tables, it is clear that adding algae extract had a clear effect in increasing the percentage of nutrients in the leaves, and this increase may be due to the fact that these extracts contain

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major and minor nutrients that are directly absorbed by the leaves and thus increase their percentage in the plant[10, Shekhar et al. (2012) and . The organic acids contained in the algae extract, in addition to its content of growth regulators and its content of major elements, including nitrogen, which is considered a rich source of phosphorus, increase the plant's ability to absorb nutrients such as nitrogen, phosphorus, potassium, iron and magnesium, which results in increased growth of the plant's vegetative and root system [9] In addition, adding seaweed extract has caused an increase in the percentage of carbohydrates, which plays a role in improving the growth of seedlings and increasing their chlorophyll content. Seaweed extract also leads to an increase in the rate of photosynthesis, which in turn leads to an increase in the rates of carbohydrates in the plant. The reason may be due to the role of organic fertilizers and the nutrients they contain, as nitrogen is the main component of protein, and potassium indirectly affects the biosynthesis of protein by activating many enzymes responsible for protein synthesis, and many of these enzymes participate in nitrogen metabolism [14] potassium is an essential ion in protein metabolism, and increasing the percentage of potassium, which is an ionic and enzymatic regulator of many physiological processes within the plant [15] is important in the mechanism of closing and opening stomata and regulating membrane permeability and has an important role in maintaining a reduction in osmotic potential within the cytoplasm of the plant cell and encourages the absorption of water and nutrients needed by the plant and then stimulates photosynthesis enzymes, which increase total protein production. The reason for the decrease in catalase and peroxidase enzymes may be due to the effect of adding seaweed extract and its ability to improve plant growth, and this is what was shown by the results of this study of improving all components

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