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Response of *Citrus aurantium* L. orange seedlings to the addition of NPK complex nanofertilizer and humic and their effect on vegetative traits and nutrients.

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Abstract

The study was carried out at University of Thi-Qar on 02.02.2023; the experience finished on 15.06.2023 on Citrus seedlings at one year age, by three seedlings for each experimental unit. Thus the number of seedlings was 81 at anvils plastic, which was filed by soil and peatmoss at a ratio of 3:1. The experiment included fertilizer treatment Nano NPK which spray on plant leaves at concentrations (0, 0.5, 1) g. l, by three additions at 15 days period between each addition. Humic acid was added to the Spray the plant three times (0, 1, 2) g. The experiment was carried out by three replication according to Randomized Complete Block Design (RCBD). The results were compared according to less difference test than 5 % possibility.

Spraying treatments with nano-fertilizer npk showed a positive effect on most of the vegetative and chemical indicators of orange seedlings. The spraying treatment at a concentration of 1 gm l-1 was significantly superior by recording the highest rate for the number of plant leaves, stem thickness, leaf content of carbohydrates, and leaf content of the nutrients calcium, magnesium, and sulfur. It reached 61.78 leaves. Seedling-1, 7.77 cm, 10.21%, 155.62%, 0.268%, 38.046%, respectively.

The average spray treatments with Humic showed a positive effect on most of the vegetative and chemical indicators of Citrus aurantium L. orange seedlings. The spray treatment at a concentration of 1 gm L^{-1} was superior in recording the highest rates for the number of plant leaves and stem thickness, as they reached 64.78 and 8.77, respectively. The spraying treatment at a concentration of 1 gm L^{-1} also excelled by recording the highest rate for the leaf content of carbohydrates and the leaf content of the nutrients calcium, magnesium and sulfur, reaching 10.01%, 156.53%, 0.257%, 36.06%.

The interaction treatments between the two study factors gave a positive, significant effect on most of the vegetative and chemical traits studied. The interaction treatment between the concentration of 1 gm L^{-1} of nano-NPK and 1 gm L^{-1} Humic recorded the best results significantly for growth indicators.

I. INTRODUCTION

The bitter orange plant returns *Citrus aurantium L.* belongs to the citrus genus Citrus, the Rutaceae family, which grows in tropical and subtropical regions, and India is considered the original homeland of aurantium (Al-Khafaji et al., 1990).

Most citrus species are widespread in the central regions of Iraq due to their suitability to the prevailing environmental conditions. The number of fruit-bearing citrus trees is approximately 7,768,290 million trees, with a production of 176,117 tons (Central Bureau of Statistics, 2020).

Citrus fruits are of great importance among other fruit trees due to their nutritional, environmental, economic and medical importance, as their fruits are a rich source of vitamins, especially vitamin C, in addition to being rich in the mineral elements necessary to build the human body (Ahmed and Dawoud, 2020).

The foliar fertilization method is efficient and effective in feeding plants through the vegetative parts, as well as providing the plant with nutrients in a homogeneous manner (Brayan, 1999).

It is important to pay attention to adding chemical fertilizers to various plants because of their role in plant growth and development, and even extends to the various biological reactions that occur within the plant. Excessive use of various chemical fertilizers is considered one of the reasons for the deterioration of the environment and soil. Nanofertilizers are the latest and most technically advanced in supplying plants with nutrients. Mineral and compared to chemical fertilizers ،Hence, the efficiency of fertilizer use improves (Subbarao et al., 2013). Where old chemical fertilizers are replaced with nano-fertilizers that are efficient and environmentally friendly in nature, the main use of adding fertilizers is the rapid absorption of nutrients while giving the best and fastest yield.

The organic matter added to the plant also improves the physical properties and structure of the soil, increases the stability of its aggregates and its ability to retain water, and is a source of nutrients necessary for plant nutrition. Humic acid, after being added to the soil, increases the absorption of nutrients by the plant. Humic acid is a transport medium for nutrients from the soil to the plant, as it affects plant growth through its effect on increasing the growth of the root system (Chen and Aviad, 1990).

Due to the lack of previous studies on the effect of spraying with NPK and humic acid on orange seedlings in Dhi Qar Governorate, this study was conducted with the aim of knowing the effect of spraying with NPK and humic acid individually or in combination in improving the chemical characteristics of orange seedlings with the aim of obtaining strong-growing, viable seedlings. To vaccinate them, as well as reduce the environmental and economic impact resulting from the use of traditional fertilizers

This study targeted:

1- Knowing the effect of treating orange seedlings with different concentrations of NPK nanofertilizer and the possibility of some changes occurring through which it is possible to give better characteristics to the plant's vegetative growth.

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2 - Detect the changes that occur when humic acid is added to the vegetative growth characteristics of plants

II. MATERIALS AND METHODS

The experiment was carried out in Thi Qar in 2023 on one-year-old orange seedlings, which were chosen as homogeneous in growth and size as possible. The seedlings were brought from one of the private nurseries in Karbala Governorate. After that, the seedlings were transferred to appropriate sized plastic anvils (containing 10 kg) and a planting medium consisting of zumig and peat moss was used in a ratio of 1:3 respectively, and samples were taken from the medium before the start of the experiment to conduct laboratory analyzes on it (Table 1)

Table (1) some physical and chemical characteristics of the soil in which seedlings are planted

Analysis type	Analysis' results	Measuring unit
Sand	776	$g.kg^{-}1$
Silt	118	
Clay	106	
soil texture	Sandy loam	
PH	8.00	
EC	2.37	$ds.\ m^{-1}$

A. Study factors

The first factor :

NPK Nano-fertilizer three concentrations (0, 0.5, 1) $g.\ l^{-1}$, NPK Nano-fertilizer contains balanced NPK elements 20:20:20: It is a product manufactured by Al-Khadra Company for the production of chelating Nano fertilizers in Iran and was used as a spray in the early morning until total wetness, at a rate of times sprays on the leaves on 15/02, 01/03, and 16/04/2023.

The second factor:

Humic acid Humic was added in three concentrations (0, 1, 2) g.l and added to Spray the plant three times on 20/02. ٠٥/٠٣/and20 /3/2023.

B . Treatments and experimental design:

This experiment was carried out on 81 seedlings of Cutrs seedlings of homogeneous vegetative growth as much as possible. Two factors were used in the experiment: NPK and humic Nano-fertilizer with three concentrations of each. Thus, the treatments were thus a factorial experiment with two factors, 3×3=9 treatment within the design of randomized complete block (RCBD) and three replications with three seedlings for each experimental unit. The number of seedlings included in the experiment is 81. The

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study results were statistically analyzed, and the averages were compared according to the least significant difference (LSD) test at a probability level of 0.05 (Al-Sahoki and Waheeb, 1990).

C. Studied characteristics:

1- Number of leaves (sheet. Plant $^{1-}$)

The number of total leaves of plants was calculated at the end of the experiment by counting the number of leaves per branch

2- Leg diameter (mm)

The diameter of the seedling was measured using the vernear foot.

3- Determination of Total Carbohydrate:

The total carbohydrate content was estimated by the (Hedge and Hofreiter) method. (0.2 gm) of the sample to be measured is added to it (25ml) of perchloric acid (1N) and placed in a test tube, then the tubes are placed in a water bath at a temperature of (60) for (30 minutes) after which the sample is filtered using filter papers. ((1 ml of the filtrate and add to it (9 ml) of distilled water to complete the volume to (10 ml) in a volumetric bottle.

1 ml) is taken from the last and a concentration of phenol (5% + 5 ml)), concentrated sulfuric acid, is added to it and left until it cools. It is measured at a wavelength (490 nm) with a spectrophotometer that prepares several concentrations of glucose (10, 20, 30, 40, 50, 60, 70) and the absorbance of the above readings is recorded (to make a calibration curve).

4- Estimation of some nutritional elements in the leaves:

The Keldahl method was used to estimate the percentage of nitrogen in the models, based on the method mentioned by (van Dijk, D 2000) and others. By taking a known weight from the model, approximately 0.2 grams, placed in a beaker, then adding (5 ml) of concentrated sulfuric acid to the sample. An appropriate amount of a mixture of potassium sulphate and copper sulphate was added, and the digestion process was carried out by heating the contents, and after the end of digestion, the mixture was transformed into a clear liquid of a pale blue color. The liquid was transferred quantitatively to the distillation flask of the Kjeldahl device, which contains a concentrated solution (40%) of sodium hydroxide and is connected to the distillation flask, condenser ending with a test tube immersed in a receiving flask containing a known volume of boric acid (20%), plus drops of methyl red indicator. And the dye (bromocresol blue), then the estimation flask is heated until the amount of distilled liquid collected in the flask reaches about (25 ml), then the collected liquid is flushed with hydrochloric acid (0.1 standard), and a control solution (Planck) of the chemicals above, except for the model, is prepared and the percentage is calculated. Protein according to the following equation.

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III. RESULTS

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

The results of Table (2) indicate that the study factors and their interactions had a significant effect on increasing the number of leaves of orange seedlings. Spraying the seedlings with nano-NPK had a significant effect on increasing the number of leaves of the seedlings with increasing spray concentration, as the spraying treatment with a concentration of 1 gm L^{-1} was significantly superior, recording a higher The average number of leaves per seedling was 61.78 leaves. Seedling^{-1} compared to the comparison treatment, which recorded the lowest number of leaves per seedling, amounting to 57.11 leaves. Seedling^{-1}.

As for spraying with Humic Assad, the results of the same table indicate a significant effect of spraying treatments in increasing the number of seedling leaves. The concentration of 1 gm. liter^{-1} recorded the highest number of seedling leaves, amounting to 64.78 leaves. Seedling-1 compared to the comparison treatment, which gave the lowest number of leaves, amounting to 44.78 leaves. Seedling$^{-1.}$

As for the interaction coefficients between nano-NPK and humic acid, the results of the same table indicate a clear significant increase in the number of leaves of orange seedlings. The spraying treatment with nano-NPK at a concentration of 1 gm .l^{-1} and humic acid with a concentration of 2 gm l^{-1} recorded the highest number of leaves for seedlings, reaching 73.33 leaves. Seedling^{-1}, which differs significantly from the spraying treatment with nano-NPK at a concentration of 50 mg. L^{-1} and humic acid at a concentration of 0 g. L^{-1}, which recorded a number of leaves for seedlings of 42.33 leaves. Seedling^{-1}.

Table (2) Effect of nano-NPK, humic acid, and the interaction between them on the number of leaves of orange seedlings (leaves. Seedling^{-1})

NPK Nano Fertilizer Concentrations	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	43.00	73.33	55.00	57.11
N1	42.33	58.00	65.00	55.11
N2	49.00	63.00	73.33	61.78
Humic acid level	44.78	64.78	64.44	
LSD 0.05				
N=1.653	H= 1.653		N*H=2.863	

2- The thickness of the leg is cm

The results of Table (3) indicate that the study factors and their interactions had a significant effect on increasing the stem thickness of orange seedlings. Spraying seedlings with nano-NPK had a significant

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effect on increasing the stem thickness of seedlings with increasing spray concentration, as the spraying treatment with a concentration of 1 gm L^{-1} was significantly superior, recording a higher The average stem thickness of seedlings was 7.77 cm compared to the comparison treatment, which recorded the lowest seedling thickness of 6.22 cm.

As for spraying with humic acid, the results of the same table indicate a significant effect of spraying treatments on increasing seedling stem thickness. The concentration of 1 gm L^{-1} recorded the highest rate of seedling stem thickness of 8.77 cm compared to the comparison treatment, which gave the lowest thickness of 6.03 cm.

As for the interaction coefficients between nano-NPK and humic acid, the results of the same table indicate a clear significant increase in the stem thickness of orange seedlings. The spraying treatment with nano-NPK at a concentration of 1 gm l^{-1} and humic acid with a concentration of 1 gm l^{-1} recorded the highest stem thickness of seedlings reaching 9.40. cm, which differs significantly from the spray treatment with nano-NPK at a concentration of 0 g. L^{-1} and humic acid at a concentration of 0 gm L^{-1}, which recorded the stem thickness of the seedlings reaching 5.26 cm.

Table (3) Effect of nano-NPK and humic acid and their interaction on stem thickness of orange seedlings (cm)

NPK Nano Fertilizer Concentrations	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	5.26	7.66	6.93	6.22
N1	7.36	9.26	6.03	7.55
N2	5.46	9.40	8.46	7.77
Humic acid level	6.03	8.77	7.14	
$LSD_{0.05}$				
N=0.378	H= 0.378		N*H=0.655	

3- Carbohydrate content of leaves%

It is clear from the results of Table (4) that spraying orange seedlings with nano-NPK has a significant effect on the percentage of carbohydrates in the leaves. The concentration of 1.0 gm L^{-1} NPK nano was superior to the rest of the treatments, resulting in the highest average percentage of carbohydrates in the leaves, amounting to 10.21%, while the percentage of carbohydrates in the comparison treatment was 8.78%.

As for the effect of spraying with humic acid on the carbohydrate content of the leaves, the results of Table (4) showed that there was a significant difference. The 2 gm l^{-1} treatment excelled in giving the highest average of carbohydrates in the leaves by 10.01, while the lowest percentage was in the comparison treatment, as it was 10.01. %8.98.

The interaction between nano-NPK and humic acid has a significant effect on the percentage of carbohydrates in the leaves. The results of table (4) showed that the treatment of 1 gm l^{-1} nano-NPK and 1 gm $^{l-}$humic acid was superior, and the highest percentage of carbohydrates reached 10.46%, while it was the lowest. The percentage of carbohydrates in the leaves during the comparison treatment reached 8.22%.

Table (4) Effect of nano-NPK, humic acid, and their interaction on the percentage of carbohydrates in orange seedlings

NPK Nano Fertilizer Concentrations	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	8.22	8.61	9.52	8.78
N1	8.97	9.22	10.07	9.42
N2	9.75	10.46	10.43	10.21
Humic acid level	8.98	9.43	10.01	
LSD 0.05				
N=0.134	H= 0.134	N*H=0.233		

4- Calcium in leaves(ca %)

It is clear from the results of Table (5) that spraying orange seedlings with nano-NPK has a significant effect on the percentage of calcium in the leaves. The concentration of 1 gm L^{-1} NPK nano was superior to the rest of the treatments, resulting in the highest percentage of calcium in the leaves, reaching 155.62%, while the percentage of calcium in the comparison treatment was 131.93%.

As for the effect of spraying with humic acid on the calcium content of the leaves, the results of Table (5) showed that there was a significant difference. The 2 gm l^{-1} treatment excelled in giving the highest percentage of calcium in the leaves at 156.53, while the lowest percentage was in the comparison treatment, at 135.21. %.

The interaction between nano-NPK and humic acid has a significant effect on the percentage of calcium in the leaves. The results of table (5) showed that the treatment of 1 gm l^{-1} nano-NPK and 1 gm l^{-1} humic acid was superior, and the highest calcium percentage reached 161.97%, while it was the lowest. The percentage of calcium in the leaves during the control treatment reached 124.27%.

Table (5) Effect of nano-NPK, humic acid, and their interaction on the percentage of calcium in orange seedlings (%)

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NPK Nano Fertilizer Concentrations	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	124.27	127.60	143.93	131.93
N1	133.00	139.00	151.47	141.16
N2	148.37	161.97	156.63	155.62
Humic acid level	135.21	142.86	156.53	
$LSD_{0.05}$				
N=1.048	H= 1.048	N*H=1.816		

6- Magnesium in leaves(Mg %)

The results of Table (6) indicate that the study factors and their interactions have a significant effect in increasing the magnesium percentage of orange seedlings. Spraying the seedlings with nano-NPK had a significant effect in increasing the seedling magnesium percentage with increasing spray concentration, as the spraying treatment with a concentration of 1 gm L^{-1} was significantly superior with a higher record. The average magnesium percentage for seedlings was 0.268% compared to the comparison treatment, which recorded the lowest magnesium percentage for seedlings, which was 0.224%.

As for spraying with humic acid, the results of the same table indicate a significant effect of spraying treatments on increasing the percentage of magnesium in seedlings. The concentration of 2 gm l^{-1} recorded the highest percentage of magnesium in seedlings, reaching 70.25%, compared to the comparison treatment, which gave the lowest increase, amounting to 0.231%.

As for the interaction coefficients between nano-NPK and humic acid, the results of the same table indicate a clear significant increase in the magnesium percentage of orange seedlings. The spraying treatment with nano-NPK at a concentration of 50 mg l^{-1} and humic acid with a concentration of 1 gm l^{-1} recorded the highest magnesium percentage for seedlings, reaching 0.277. %, which differs significantly from the spraying treatment with nano-NPK at a concentration of 0 ml L^{-1} and humic acid at a concentration of 0 g L^{-1}, which recorded a magnesium percentage for seedlings of 0.209%.

Table (6) Effect of nano-NPK, humic acid, and their interaction on the percentage of magnesium in orange seedlings (%)

NPK Nano Fertilizer Concentrations	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	0.209	0.218	0.246	0.224
N1	0.227	0.235	0.256	0.239

N2	0.257	0.277	0.270	0.268
Humic acid level	0.231	0.243	0.257	
LSD 0.05				
N=0.003	H= 0.003	N*H=0.006		

7- Sulfur in leaves (S%)

The results of Table (6) showed that there were significant differences in the percentage of sulfur in the leaves of orange seedlings when sprayed with nano-NPK and humic acid. The results of spraying 1 gm l^{-1} of nano-NPK by giving it showed the highest percentage of sulfur in the leaves, reaching 38.046%, while the percentage of sulfur was the lowest. In the leaves of orange seedlings when treated without addition, it reached 26.35%.

The results in Table (7) indicated that there were significant differences between the treatments when spraying Humic Asad orange seedlings in the percentage of sulfur in the leaves, as the 2 gm l^{-1} treatment excelled in giving it the highest percentage of sulfur in the leaves, reaching 36.06%, thus superior to the comparison treatment, which gave 27.92. %.

As for the effect of the interaction between the study factors, the results in the same table showed a significant increase in the percentage of sulfur in the leaves. Spraying 1 gm. L^{-1} nano NPK and 1 gm. L^{-1} humic acid resulted in the highest sulfur percentage reaching 41.40%, while the lowest sulfur percentage. In the papers, the result of the comparison treatment for both nanoNPK and humic acid was achieved at 22.16%.

Table (2) Effect of nano-NPK, humic acid, and their interaction on the sulfur percentage in orange seedlings (%)

NPK Nano Fertilizer Concentrations	Humic acid concentrations			Fertilization rate
	H0	H1	H2	
N0	22.16	24.53	32.36	26.35
N1	26.67	29.26	36.77	30.90
N2	34.93	41.40	39.04	38.046
Humic acid level	27.92	31.72	36.06	
LSD 0.05				
N=0.483	H= 0.483	N*H=0.837		

IV. DISCUSSION

Researchers have turned to finding modern technical methods and methods for the purpose of adopting them in supplying plants with the necessary nutrients for their continued growth and achieving quantitative and qualitative improvement in their production, by reducing or eliminating the obstacles faced by nutrient acids in the soil that reduce their readiness for the plant. As a result of the above, it becomes clear to us that The increase in leaf area was the result of spraying with nanofertilizers, especially nitrogen, which has a major role in the process of cell division and elongation. Especially since it is mainly involved in the synthesis of protein and amino acids (Wample et al., 1991), and the deficiency of the element leads to a decrease in the rate of protein synthesis in addition to many necessary compounds, and also that phosphorus has a direct effect on division and increased branching, which causes an increase in the number of leaves and the number of branches. And leg fish (Abdel Qader et al. 1982). Potassium has a role in encouraging the growth of meristematic tissues, in living cell division and the process of carbon assimilation, as well as the transfer of materials resulting from this process and activating the enzymatic system.

The increase that we obtained when adding humic in most vegetative growth characteristics may be due to humic acids in increasing the various biological and physiological activities necessary for plant growth. Dantas and others (2007) mentioned that humic acid contains a group of quinines that work

As a hydrogen receptor, which increases the activity of enzymes, it also has a major role in the processes of photosynthesis and respiration. Humic acid has an important role in interacting with the phospholipid compounds present in the structure of cell membranes, and it works inside the cell, which increases its permeability. These compounds act as a carrier for transporting nutrients from outside. Cell membranes, thus increasing the absorption of water and nutrients

Humic acid is safe and highly soluble in water, easy to add, quickly effective, and does not leave any harmful effects on humans or plants. Humic acids also reduce the problems of excess salinity, which causes toxicity. This was confirmed by adding humic acid to the soil, as it reduced the harmful effects of salinity on growth. Citrus seedlings thus improved the studied characteristics of vegetative growth and seedling nutrients.

Humic acid also enters the plant as a complementary source of polyphenols, which act as a respiratory chemical mediator that leads to an increase in the biological activity of the plant, as the effectiveness of the enzymatic system increases and thus cell division increases. Humic acid increases the exchange capacity of positive ions and lowers the pH values, which stimulates the growth of organisms, especially fungal ones.

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