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Estimation of active substances and compounds in orange seedlings *Citrus aurantium* L when spraying with nanofertilizers and humic acid

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Abstract

The experiment was conducted at the research station of the Faculty of Agriculture and Marsh of Thi Qar University during the growing season (2022-2022). Orange seedlings were obtained. Citrus aurantium, one year old, grown as uniformly as possible, from a nursery in Diyala governorate in 2022, and the seedlings were transplanted into 10 kg plastic pots (26 cm diameter) filled with a growing medium mixed with soil and peat moss. The plant care process was carried out in a similar manner throughout the study period, including pruning, weeding, fertilization and irrigation. The study was conducted as a two-factor factorial experiment using a randomized complete block design (R.C.B.D.). The first factor represented the spraying of composite nanofertilizer NPK at three concentrations (1, 0.5, 0) g.L-1, and the second factor represented the spraying of humus at three concentrations (0, 1, 2,) g.L-1. The interval between one spraying and the next was 3 sprayings with an interval of two weeks. The results of the study can be summarized as follows:

The spraying treatment of nanofertilizer NPK showed a positive effect on most of the active ingredients in bitter orange plants. The spray treatment with 100 mg L-1 concentration significantly outperformed the total sugar, naringenin, limonene, terpenes, and pyran compounds by 0.793%, 59.00%, 2.967%, 15.468%, and 7.954%, respectively. The average organic fertilizer spray treatment showed positive effects on most nutritional and chemical parameters of bitter orange seedlings. Citrus aurantium. The spray treatment with 2 g L-1 concentration had a better effect, with the highest proportions of total sugar, naringenin, limonene, terpenes, and pyran compounds, which were 0.760%, 55.28%, 2.772%, 15.086%, and 7.903%, respectively. The interaction coefficient between the two examined factors had a significant positive effect on most of the examined phytochemical and chemical properties. The interaction coefficient between 100 ml L-1 nano-NPK concentration and 2 g L-1 humus concentration recorded the best results for the active ingredient indicators such as naringenin, limonene, terpenes, and pyrans..

1. Introduction

Citrus fruits belong to the Rutaceae family and are characterized by the presence of aromatic oil glands in most parts of the plant, which distinguishes it from other fruits. The bitter orange plant Citrus aurantium L. belongs to the Rutaceae family, the genus Citrus, and grows in tropical and subtropical regions. India, being their homeland, is one of the main producers of various citrus fruits because they are abundant in yield and are compatible with most citrus fruits. Moreover, it is a good producer for moderate and heavy soils (Salman, 1988).

Most types of citrus fruits are common in the central region of Iraq as they are suitable for the local environmental conditions. Number of citrus trees 776829 million, production 176117 tons (Central Statistical Organization, 2020).

From an economic perspective, citrus fruits contribute to satisfying many of the increasing desires of the consumer population as one of the materials that undergo some manufacturing processes to provide food, as citrus trees and their products are considered a raw material in industry. The trunks are used to roof houses and make bridges and canals, and their wood is used for home furniture. Some pharmaceutical components are extracted from their roots, while

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their fruits are used in various industries such as extracting oils. For example, aromatic oils extracted from the peel of fruits are used in the soap industry, the pesticide industry, and in perfuming some types of foods, sweets, and some types of jam. They are widely used in perfuming carbonated water to give it a natural flavor (Ahmed and Daoud, 2020).

Despite the multiple causes of the food insecurity problem, and despite the fact that humans have made and are still making strenuous efforts to solve the food shortage problem, such as using chemical pesticides that consume a large part of agricultural crops, or expanding the cultivation of arable desert lands, or resorting to genetic engineering modification techniques with the aim of developing new strains of plants with higher productivity, and other solutions, the use of agricultural fertilizers is one of the basic pillars of agricultural development processes and thus food production, as their use has had a dazzling impact on agricultural production and has been a basic element in achieving agricultural renaissance worldwide.As we all know, fertilizers usually compensate for the lack of soil fertility on the one hand and the lack of arable land or its stability on the other hand, especially considering the problem of population growth. Fertilizers are essential for human food production, but the use of fertilizers in agriculture seems to have become a double-edged sword, with the need to strengthen production and ensure food security, but also facing the risk of excessive or incorrect use. Therefore, compared with chemical fertilizers, nanofertilizers are the latest and most technologically advanced means of providing mineral nutrients to plants, thereby improving fertilizer utilization efficiency (Subbarao et al., 2013). Although old chemical fertilizers are being replaced by efficient and environmentally friendly nanofertilizers, the main purpose of adding fertilizers is to quickly absorb nutrients and obtain the best and fastest yield. Adding humic acid to the soil can increase the absorption of nutrients by plants because it acts as a medium for transporting nutrients from the soil to the plants, especially in drought conditions. It can also increase and improve the intensity of root growth, increase the protein content of plants and increase the number of beneficial microorganisms in the soil. Humic acid decomposes soil and improves its physical, chemical and biological properties by breaking down clay particles and increasing the water retention capacity of the soil. Humic acid promotes the development of chlorophyll, sugars, and amino acids, and aids in the photosynthesis process. Their role is similar to that of auxin in cell division, promoting plant growth. It also promotes seed germination, and its role is the same as the formation of plant roots, as it transports nutrients and water to the seeds, stimulating plant growth. Given the lack of previous research on the effects of spraying nanofertilizers and humic acid on bitter orange seedlings in Dhi Qar Province, this study aimed to investigate the effects of spraying NPK and humic acid alone or in combination on improving bitter orange seedlings. Active ingredients in bitter orange seedlings to obtain vigorous growing seedlings suitable for grafting..

II . MAT ERIALS AND METHODS

3-1: Study site

The experiment was conducted in the Saran mulch nursery of the Department of Horticulture and Landscape Engineering/College of Agriculture and Wetlands, Thi Qar University during the growing season-2022, some one-year-old Citrus aurantium seedlings were selected for this study, in which an agricultural growing medium consisting of soil and peat moss in a ratio of 1:2 was used, and samples were randomly taken from the river mixture and analyzed in the laboratory to determine some chemical and physical characteristics of the soil. (1).

Table (1) Some chemical and physical properties of the soil mixture

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Value	Unit of measurement	Adjective.
7.1	------	Ph
2.11	Ds .m$^{1=}$	EC
Sandy Mxture	Silty loam	Tissue
70.24	Mg L^{1-}	Sandy.
14.44	Mg L^{1-}	Green
15.42	Mg L^{1-}	Clay
2.89	Mg L^{1-}	N
3.55	Mg L^{1-}	P
4.54	Mg L^{1-}	K

3-2: Seedling preparation and service

Orange seedlings were obtained from a nursery in Diyala Governorate in 2022. They were approximately one year old, uniformly grown, and planted in polyethylene plastic bags. Seedlings were transplanted into 10 kg plastic pots (26 cm diameter). Plant servicing, including pruning, weeding, fertilization, and irrigation, was performed in a similar manner throughout the study period..

A. Study factors

The first factor

Included spraying the green group of seedlings with the nano-NPK compound at three concentrations: 0, 50, and 100 mg/L

The second factor

Included spraying the green group of seedlings with Humic Acid at three concentrations: 0, 1, and 2 g/L

B . Treatments and experimental design:

The experiment was conducted on 81 Cutrs seedlings with vegetative growth as uniform as possible. Two factors were used in the experiment: NPK and humus nanofertilizer, three concentrations each. Therefore, the treatment was a two-factor experiment with 3×3=9 treatments within the design (CRBD) and three replications of three seedlings per experimental unit. The number of seedlings included in the experiment was 81, and the results of the study were statistically analyzed and tested according to the least significant difference test of 0.05. (Al-Rawi,, Khalaf. 2000).

C. Studied characteristics:

Indicators of active compounds:

1- Estimation of the percentage of total sugars in the leaves (mg. 100 ml^{-1}):

The method is to take 2g of wet sample, place it in a test tube, add 80ml of 80% ethanol, and determine the total sugar percentage. Place the sample in a 60°C water bath for 60min, repeat 3 times, and centrifuge at 3000rpm for 15min each time. Collect the clear solution in a volumetric flask and make up the volume to 25ml by adding perchloric acid. Add 5ml of concentrated sulfuric acid to 1ml of 5% phenol solution. Use a spectrophotometer to read the absorbance of the solution at a wavelength of 490nm. Close the standard solution with known glucose concentration and draw a standard curve, and then the absorbance value falling on the standard curve can determine the sugar concentration in the sample..

2-Leaf content of medicinal and active compounds:

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The samples were collected in the early morning of April 2, 2022, and fresh leaves were placed in transparent polyethylene bags. The bags were labeled and placed directly in a cooler with ice to prevent the samples from wilting as much as possible. The samples were sent directly to the laboratory of the Ministry of Science and Technology in Baghdad and stored at 4°C until the samples were analyzed.

The medicinal compounds naringenin, limonene, terpenes and bayan were measured using a high performance liquid chromatography (HPLC) according to the method (Lenchyk, 2015) and according to the equation.

Sample concentration (µg.L-1) = (sample area/standard solution area) × standard solution concentration × number of dilutions (4).

III . RESULTS

1- Total soluble sugars

The results in Table (2) show that the investigated factors and their interactions have a significant effect on increasing the soluble sugar of orange. With the increase in spray concentration, the spraying of nano-NPK has a significant effect on increasing the soluble sugar of seedlings. The spray treatment with a concentration of 001 mg·L-1 has the highest average soluble sugar of 0.793%, while the control treatment with the lowest soluble sugar is 0.602%.

For humic acid spraying, the results in the same table show that the spraying treatment has a significant effect on increasing soluble sugar, and the 2 g L-1 concentration produces the highest soluble sugar, which is 0.760, an increase of 0.617% compared with the lowest level treatment of 87.56 cm.

As for the interaction coefficient between nano-NPK and humic acid, the results in the same table show that the soluble sugar of bitter orange seedlings increased significantly. The soluble sugar value of the spray treatment with nano-NPK at a concentration of 100 mg·L-1 and humic acid at a concentration of 1 g·L-1 was the highest, reaching 0.843, which was significantly different from the nano-NPK spray treatment. When the concentration is 0 ml L-1 and the humic acid concentration is 0 g L-1, the soluble sugar value is 0.520%.

Table (2) The effect of nano NPK and humic acid and their interaction on the total soluble sugars of bitter orange seedlings (%)

Fertilization rate	Humic acid concentrations			Nano NPK Fertilizer Concentrations
	H2	H1	H0	
0.602	0.706	0.580	0.520	N0
0.672	0.760	0.646	0.610	N1
0.793	0.813	0.843	0.723	N2
	0.760	0.690	0.617	Humic acid rate
05		.0	LSD	
N*H=0.384		H=0.222		N=0.222

2- Naringenin

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The results in Table (3) show that when bitter orange seedlings were sprayed with different concentrations of Nano-NPK, there were differences in the response of the content of naringenin compounds in the leaves. The leaves treated with 100 mg L-1 had the highest naringenin content of 59.00%, and the leaves treated with 0 ml L-1 had the lowest naringenin content of 41.02%.

As for the effect of humic acid spraying, the results in the same table showed that the spraying treatment had a significant effect compared with the control treatment, in which the naringenin content was the lowest at 43.69%, and the increase was proportional to the increase in the spraying concentration. The highest naringenin content was 55.28% when the concentration was 2 g L-1.

The interaction between the studied factors had different effects on increasing the naringenin content in the leaves, in which the 100 mg L-1 Nano-NPK and 1 g L-humic acid treatment showed a clear advantage as it provided the highest naringenin content, which was 63.97% compared with the control treatment..

Table (3) Effect of nano NPK and humic acid and their interaction on naringenin in bitter orange seedlings (%)

Fertilization rate	Humic acid concentrations			Nano NPK Fertilizer Concentrations
	H2	H1	H0	
41.02	48.77	38.08	36.21	N0
47.52	56.74	44.24	42.17	N1
59.00	60.33	63.97	52.70	N2
	55.28	48.76	43.69	Humic acid rate
05		.0 LSD		
N*H=1.117		H=0.645		N=0.645

3- Limonene

The results in Table (4) show that after spraying bitter orange seedlings with different concentrations of Nano-NPK, there are differences in the response of leaf limonene content, among which the concentration of 100 mg·L-1 is better, with the highest content. The limonene content is 2.967%, while the lowest leaf limonene content is 1.901% in the 0 ml L-1 treatment.

The results in the same table also show that spraying bitter orange seedling leaves with humic acid has a significant effect on increasing the limonene content of leaves. The 2 g·L-1 spray treatment is characterized by the highest limonene content, reaching a value of 2.772%, while the treatment (no spraying) gives the lowest content of this property, which is %.

The interaction between the studied factors has different effects on increasing the limonene content in leaves, because the treatment with 100 mg L-1 Nano-NPK and 1 g L-humic acid showed a clear advantage in providing the highest limonene content, which was reduced by 3.140% compared with the control treatment..

Table (4) Effect of nano NPK and humic acid and the interaction between them of limonene in the leaves of bitter orange seedlings (%).

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Fertilization rate	Humic acid concentrations			Nano NPK Fertilizer Concentrations
	H2	H1	H0	
1.901	2.256	1.833	1.613	N0
2.390	2.983	2.203	1.983	N1
2.967	3.076	3.140	2.686	N2
	2.772	2.392	2.094	Humic acid rate
05		.0	LSD	
N*H=0.1937		H=0.1118		N=0.1118

4- Terpenen percentage in leaves (%)

The results in Table (5) show that spraying nano-NPK on bitter orange seedlings has a significant effect on the terpene content in leaves. The 100 g L-1 nano-NPK concentration is superior to other treatments, and its leaf terpene content is the highest, which is 15.468%, while the terpene content of the control treatment is 14.209%.

As for the effect of spraying humic acid on the terpene content of leaves, the results in Table (5) show that the difference is significant. The terpene content in leaves of the 2 g L-1 treatment is the highest, reaching 15.086%, while the proportion in the control treatment is the lowest at 14.458%.

The interaction between nano-NPK and humic acid has a significant effect on the terpene content in leaves. The results in Table (5) show that the 100 mg L-1 nano-NPK and 1 g L-humic acid treatments are more effective, with the highest terpene ratio of 15.730%, while the proportion of terpene in leaves is the lowest for the comparison treatment, i.e. 13.787%.

Table (5) Effect of nano NPK and humic acid and their interaction on the percentage of terpenes in the leaves of bitter orange seedlings (%).

Fertilization rate	Humic acid concentrations			Nano NPK Fertilizer Concentrations
	H2	H1	H0	
14.209	14.863	13.977	13.787	N0
14.637	14.834	14.603	14.463	N1
15.468	15.550	15.730	15.123	N2
	15.086	14.770	14.458	Humic acid rate
05		.0	LSD	
N*H=0.307		H=0.177		N=0.177

5- Percentage of pyrin in leaves (%)

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The results in Table (6) show that the pyridine content in the leaves of bitter orange seedlings was significantly different when spraying nano-NPK and humic acid. The results of spraying 100 mg·L-1 nano-NPK showed that the pyridine content in the leaves of bitter orange seedlings was the highest, reaching 7.954%, while the lowest pyridine content in the leaves of bitter orange seedlings appeared in the no-additive treatment, reaching 6.909%.

The results in Table (6) show that there were significant differences in the pyridine content in the leaves of bitter orange seedlings after spraying humic acid. The pyridine content in the leaves of the 2 g L-1 treatment was the highest, reaching 7.903. % Therefore, it exceeded the comparison treatment, which was 6.976%.

As for the interaction effect between the studied factors, the results in the same table showed that the content of Biani in the leaves increased significantly. The proportion of Biani in the leaves treated with 1 mg L-1 Nano-NPK and 1 g L-humic acid was the highest, reaching 8.123%, while the proportion of Biani in the leaves treated with 0 mg L-1 Nano-NPK and 1 g L-humic acid was the lowest. Obtained 0 g L-1 humic acid 6.297%.

Table (6) Effect of nano NPK and humic acid and their interaction on the percentage of biani in the leaves of bitter orange seedlings (%).

Fertilization rate	Humic acid concentrations			Nano NPK Fertilizer Concentrations
	H2	H1	H0	
6.909	7.700	6.730	6.297	N0
7.424	7.973	7.373	6.927	N1
7.954	8.037	8.123	7.703	N2
	7.903	7.409	6.976	Humic acid rate
05		.0	LSD	
N*H=0.131		H=0.075		N=0.075

IV. Discussion

Nanofertilizer nutrition plays a fundamental role in the growth and development of plants. In medicinal plants, the increase in nutrients stimulates plant growth and synthesizes essential oils and active ingredients. Table (2-3-4-5) shows that the performance of the materials tested is excellent. After the addition of nanofertilizers, the active ingredients increased significantly. This is due to their small size and increased surface area, which leads to an increase in the absorption area, thereby improving the efficiency of nutrient utilization through certain mechanisms such as targeted release and slow or controlled release, which leads to an increase in the photosynthesis process and an increase in plant nutrient production. The effect of increasing the concentration of active substances in plants can be attributed to the fact that treating plants with major nutrients increases vegetative growth and the number of leaves, which leads to an increase in carbon production and, in turn, the production of secondary carbon compounds within the plant. Nitrogen enters its composition or can help increase the formation of some enzymes responsible for the formation of these compounds. These results are consistent with those reported by Dawood (2020).

As for the superiority of the percentage of active ingredients, it is also attributed to the role of humic acid in promoting cell division and increasing the number of cells, which is reflected in the increase in the green growth of plants and thus the increase in the number of leaves. Organic matter plays an important role in improving the chemical and

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physical properties of the soil and increasing the activity of microorganisms in the soil, which increases the availability of nutrients in the soil, thereby promoting plant growth and the increase of active compounds (Gosh et al., 2004).

Due to the content of essential nutrients such as nitrogen and phosphorus in organic matter, the Improves the vegetative growth characteristics of plants by forming RNA and DNA required by cells, increasing the formation and lysis of proteins, nucleic acids and protoplasm structures. In addition to playing a role in carbon metabolism, respiration and energy supply, it also has a positive effect on the increase of active substances in leaves. This is consistent with what was said (Zeiger and Taiz, 2006).

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