ARTICLE / INVESTIGACIÓN

Effect of foliar spraying with Nano-NPK fertilizer in some growth characteristics and chemical content of some citrus rootstocks

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Abstract: The experiment was carried out in the shade house, Department of Horticulture and Landscape, College of Agriculture, Kerbala University, during the 2021 growing season to investigate the influence of foliar spraying with Nano-NPK to improve the growth indicators& nutritional content of seedlings of citrus rootstocks. The results show the citrus root Rangpur was significantly superior to Cleopatra's mandarin in most Growth characteristics and nutritional content. Also, the superiority of Nano NPK Fertilizer with a Concentration of (1.5 mg L^{-1}) by recording a significant increase in plant height, Stem diameter, Number of leaves, Leaf area, Dry weight of shoot and root, root length, leaf content of chlorophyll, percentage of carbohydrates, N, and P). In contrast, the concentration1 mg L^{-1} gave the highest proportion of (K) in the leaves. The dual interaction treatment (Rangipur root + 1.5 ml L^{-1} Nano NPK fertilizer) was superior by providing the highest rate in most of the vegetative and root characteristics and the chemical content of the leaves. The dual interaction treatment (Cleopatra mandarin + 1.5 ml L^{-1} nano NPK fertilizer) was superior by giving the highest rate in the Leaf area, dry weight of the root system & the percentage of (P) in leaves.

Key words: Nano NPK Fertilizer; Citrus, Rootstocks.

Introduction

Citrus is considered an essential evergreen tree of fruit That belongs to the family of Rutaceae, which includes 130 different genera the most important of which is Citrus, which has more than 162 species[1]. The economic, nutritional, medical and aesthetic importance of the citrus tree is the fact that the fruits are a source of pectin that enters the food industry. The peel of the fruits, flowers, and young leaves is used as a source for extracting essential oils. Citrus fruits are among the most important sources of vitamin C. Citrus fruits also contain Ca, P, K and Iron salts[2].

The cultivation of citrus spreads in the central and southern regions of Iraq, but the production hardly meets the need for local consumption except for a short period due to poor management of land and water and the lack of selection of appropriate rootstocks for grafting and neglect of pest control operations, and grafting. The Number of citrus fruit trees in Iraq is approximately 7,768,290 trees, producing up to 176,117 tons[3]. The propagation of Citrus by seeds is a method still used so far in some regions, especially tropical ones, due to the phenomenon of Poly embryonic that characterizes most citrus seeds. The sound characteristics of the grafted seedlings, as they bloom after 1-3 years of grafting, while the seed seedlings are characterized by a relatively long period of juvenility that may reach more than 7 years[1]. There is also difficulty in harvesting due to the height of these trees and the presence of thorns, in addition to some of these, some problems are related to the soil such as high levels of lime, drought, nematodes, and some are related to diseases (fungal and viral), insects and the environment[4].Choosing the right rootstocks for the purpose of grafting good varieties of Citrus has become one of the important things that must be taken into account as citrus cultivation depends mainly on choosing strong and appropriate rootstocks to obtain trees that are resistant to various environmental conditions as well as diseases that affect Citrus, whether through the root system or vegetative and obtaining the highest productivity, and among the specifications of great importance that must be met in the rootstocks is its resistance to viral diseases, one of the most dangerous of which is Tristeza, which causes the death of large numbers of citrus trees in large areas of the world[5]. One of the most important assets recently used in Iraq for graftingis the rootstock *Citrus reshni* (Cleopatra mandarin), which is from the clementine strain, which is characterized by its early production and resistance to Tristeza and Cachexia disease, and is suitable for cultivation in all types of soils[6]. As for the rootstock Rangpur, it is a hybrid resulting from the cross *Citrus* × *limonia*, which is characterized by salinity tolerance, the possibility of cultivation in clay and light soils, and its resistance to Tristeza disease that severely infects *Citrus aurantium* L., Cachexia disease and nematodes[7]. For the growth and development of rootstocks Citrus to reach the appropriate size for grafting, they must be supplied with essential nutrients to carry out the important vital and metabolic activities in plant growth, and their deficiency causes a physiological imbalance as a result of nutritional imbalance, which may affect the growth of the rootstock and budded in the future[8]. overprotecttrees and fertilizing them with macro and micro fertilizers by spraying technique on plants contributes to stimulate growth and reduces the use of fertilizer through the soil, which may cause the loss of a percentage of it through leaching or fixation between soil particles, especially in the saline soils of Iraq, and spraying with mineral elements has a remarkable effect

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in increasing the vegetative and root growth and getting rid of signs of lack of elements quickly for a number of fruit seedlings[9]. It was shown that spraying *Olea europaea* var. Kalamat seedlings with Nano NPK fertilizer at a concentration of 0.2% led to a significant increase in plant height rate, leaf area, percentage of dry matter, total chlorophyll, N, P, and K in leaves[10]. It showed that seedlings of Citrus aurantium L. with Nano NPK fertilizer at a concentration of 300 mg L^{-1} led to a significant increase in plant height, Number of branches, Number of leaves, leaves content of total chlorophyll, percentage of carbohydrates, N, P, K, iron, Zn, Mn, auxins and gibberellins[11]. It was found that spraying strawberry seedlings with nano-NPK fertilizer at a concentration of 3g.L^{-1}caused a significant increase in leaf area and leaves content of chlorophyll[12].

The aim of this study to evaluate the efficiency of the growth of the citrus rootstocks, Rangpur and Cleopatra mandarin and determine the best concentration of Nano NPK fertilizerin ,increasing the indicators of vegetative & root growth and improving the nutritional content in order to get seedlings ready for grafting in the least time and cost.

Materials and methods

Executing the experiment

Experiment carried out in lath house return to faculty of Agriculture / Department of Horticulture & Landscape / University of Kerbala / Husseiniya district During 2021the growing of season to study the effect of foliar spraying with Nano NPK fertilizer on some growth characteristics and chemical content of citrusrootstocks Rangpur and Cleopatra mandarin.

Field preparation and agricultural operations

(60 seedlings) were selected as homogeneous as possible in growth for each rootstock with a length of (30-35 cm), obtained from the Horticultural and Forestry Station / Karbala Governorate / Al-Hindiya District, planted in polyethylene bags (1.25 kg), transported on 20/2/ 2021 to 10 kg bags filled with a mixture of sandy and peatmoss at a ratio of 1:3.

A factorial experiment (2×4) was followed by The randomized completely block design {RCBD} with 3Replicates, as each replicate includes 8 treatments with 5 seedlings for each treatment, thus the total Number of seedlings is 120. The first factor was represented by the rootstock (Rangpur and Cleopatra mandarin). The second factorwas spraying with nano NPK fertilizer (10:12:12) at four concentrations (0, 0.5, 1, and 1.5 g.L^{-1}).

The seedlings were sprayed starting from 1/3/2021 until 1/7/2021 using a manual sprayer with a capacity of (2 liters) every 15 dayThe spraying of seedlings starteds in the early morning with the concentrations under study after adding (1 cm^3) Tween 20. The comparison treatment was also sprayed with distilled water only after.The seedlings were watered one day before the spraying process to increase the plants' efficiency in absorbing the sprayed substance[13]. The averages were compared according to the least significant difference (LSD) test under a probability of 0.05%[14].and the data were an-alyzed using the statistical program GenStat 2007.

Studied traits

The results were collected one month after the last treatment.

- Seedling height (cm) : Measured by a measuring tape, from the ground level to the highest point in the plant.
- Stem diameter (mm) : Measured by Vernier, for three seedlings then the mean was taken for each treatment.
- Shoot number (Shoot. $plant^{-1}$): The Number of shoot per plant was calculated and the mean was taken for each plant.
- Number of leaves: (leaves. $Plant^{-1}$) The Number of leaves per plant was calculated and the mean was taken for each plant.
- Leaf area (cm^2) : Measured by taking three leaves of different parts of each seedling and weigh and taking the tablets with an area of one cm^2 of the cut leaves. Leaves were placed in an oven at 70° C, until the weight is stableand then area of leaves were calculated according to the following formula[15].

$$\text{Leaf area} = \frac{\text{Leaf dry weight (g)} \times \text{Average of the area of the cut section (cm}^2\text{)}}{\text{Average dry weight of the the area of the cut section (g)}}$$

- Dry weight of shoot and root system (g): The roots were separated from the shoots and washed with water to remove dirt and impurities, the samples were placed in paper bags separately, and the samples were dried in an electric oven at a temperature of 70 °C, until the dry weight was established. The weight was measured using a sensitive electric scale[16].
- root length (cm): Using a tape measure from the end of the stem to the far end of the root.
- Chlorophyll content of leaves(SPAD): The chlorophyll content in leaves was estimated by using a chlorophyll meter type SPAD-502 by taking the reading for 3 leaves for each (seedling) and then taking the average for each treatment and it was measured in SPAD Unit[17].
- carbohydrates content of leaves (%): used method[18] to estimate the amount of total carbohydrates in the leaves by taking 0.2 gm of dry sample powder and for each experimental unit, adding perchloric acid (1N) solution to it and placing the sample in a water bath (60 °C) for 60 minutes and taking 1 ml of The dilute solution was added to it 1 ml of 5% phenol solution and 5 ml of concentrated sulfuric acid, then the absorption of the solutions was read by a spectrophotometer at a wavelength of 490.
- Concentration of mineral elements in leaves (%):Full-grown leaf samples were taken from the fourth to sixth leaf of the branches and then placed in (oven) at a temperature of 70 ° C Until the weight is stable

, then crushed and digested using concentrated sulfuric acid H2SO4 and HClO4 at a ratio of 1:4 for each of them, respectively, according to (19) estimated the following nutrients:

1. Percentage of total nitrogen by Micro-Kejldahl method according to (20).
2. The percentage of phosphorous: using the Spectrophotometer.
3. The percentage of potassium: using the Flame photometer.

These elements (P and K) were estimated according to the methods mentioned[21].

Results

Growth indicators

The results of Table 1. indicate that the rootstock of Citrus Rangpur was significantly superior to the Cleopatra mandarin cultivar in the rate of plant height, stem diameter, Number of shoot, Number of leaves, dry weight of the shoot and root length, which amounted to (54.77 cm, 5.26 mm, 4.25 Shoot. plant^{-1}, 77.10 leaves. Plant^{-1}, 17.23 g and 33.75 cm) respectively, while the rootstock Cleopatra Mandarin gave the highest significant increase in the leaf area characteristic, which amounted to 14.41 cm^2 compared to the Rangpur, which recorded (7.08 cm2).

Table 1. also shows that there is a significant increase in growth indicators with an increase in the concentration of Nano NPK grafting, as the concentration treatment 1.5 ml.L^{-1} exceeded by recording the highest rate in plant height, stem diameter, Number of leaves, leaf area, dry weight of the vegetative, root and root length reached (59.42 cm, 5.52 mm, 71.65 leaves. Plant^{-1}, 11.70 cm2, 18.55 g, 8.25 g and 29.67 cm) respectively compared to the comparison treatment that recorded the lowest rate of (45.92 cm, 4.28 mm and 59.50 leaves. plant^{-1}, 8.97 cm^2, 13.23 g, 5.22 g, and 23.00 cm) respectively. The treatment at a concentration of 0.5 ml. L^{-1} alsogave the highest average Number of shoot of 4.33 shoot. plant^{-1}.

Chemical indicators

Table 2. shows that the rootstock of Ranger was significantly superior in The leaves Content of Chlorophyll, carbohydrates, N & K, which recorded {61.67 SPAD, 8.25%, 2.02% and 1.38%} respectively,while the rootstock Cleopatra Mandarin recorded the highest significant increase in the percentage of phosphorus in the leaves (0.19%). The Nano NPK graftingtreatment with a concentration of 150 mg.L^{-1} caused a significant increase in the content of leaves in chlorophyll, carbohydrates, nitrogen and phosphorous, (66.43 SPAD, 9.18%, 2.18% and 0.30%) respectively and the concentration of 1 mg.L^{-1} revealed the highest significant increase of potassium, which was 1.45%, compared to the comparison treatment that recorded the lowest chemical content amounted to (56.07 SPAD, 7.33%, 1.59, 0.20 and 1.16%) respectively.

As for the interaction between the two factors of the study, the treatment of (Rangor +150 mg.L^{-1} Nano NPK fertilizer) recorded increasein the growth characteristics in the rate of plant height, stem diameter, Number of shoot, Number of leaves, dry weight of the shoot, root and root length, which amounted to (62.87 cm, 5.75 mm , 4.67 shoot. plant^{-1}, 86.00 leaves. plant^{-1}, 19.83 g, 8.23 g and 36.00 cm) respectively. while the interference treatment (Cleopatra Mandarin +150 mg.L^{-1} Nano NPK fertilizer) recorded the highest leaf area of 15.93cm^2.

As for the effect of the interaction on the chemical content of the leaves, the treatment (Rangor +150 mg.L^{-1}Nano NPK fertilize)recorded the highest content of chlorophyll, carbohydrates, N and K (68.97SPAD, 9.39%, 2.34% and 1.47%), while the interference treatment (Cleopatra Mandarin +150 mg.L^{-1} Nano NPK fertilizer) gave the highest ratio of 0.31%.

Discussion

The reason for the difference between the citrus root stocks may be for the differences to the nature of the genetic structure of the two rootstocks and their ability to absorb water and nutrients in the root area and their transfer to the leaves and improve the osmotic pressure of cells and thus improve the process of photosynthesis and growth in general[22].

The significant increase in growth characteristics and chemical content when using Nanofertilizers may be due to the unique characteristics of Nanofertilizers from the small size of the particles (1-100 nm) and the increase in the interaction surface area, which facilitates the penetration of

Treatment			Plant height (cm)	Stem diameter (mm)	Shoot number	Leaves number	Leaves area (cm^2)	dry weight of the Shoot mass (g)	length of theroot (cm)	dry weight of the root mass (g)
Rootstocks	Rangpur		54.77	5.26	4.25	77.10	7.08	17.23	33.75	6.76
	Cleopatra mandarin		50.39	4.67	3.33	51.65	14.41	15.05	20.00	6.95
LSD 0.05			2.16	0.42	0.67	4.08	0.87	1.02	1.25	N.S
	Conc.									
nano NPK Fertilizer ml.L^{-1}	0		45.92	4.28	2.83	59.50	8.97	13.23	23.00	5.22
	0.5		52.90	4.96	4.33	64.65	10.73	16.74	27.00	6.10
	1		52.07	5.09	3.83	63.70	11.57	17.55	27.83	7.88
	1.5		59.43	5.52	4.17	71.65	11.70	18.55	29.67	8.25
LSD 0.05			2.94	0.67	0.94	5.78	0.96	1.07	1.40	0.90
Rootstocks X nano NPK Fertilizer ml.L^{-1}	Rangpur	0	48.33	4.34	3.33	74.70	6.66	14.13	28.33	5.21
		0.5	54.77	5.19	4.67	76.00	6.85	16.77	36.00	5.83
		1	53.10	.545	4.33	76.70	7.32	18.20	34.67	7.83
		1.5	62.87	5.75	4.67	86.00	7.47	19.83	36.00	8.23
	Cleopatra mandarin	0	43.50	4.21	2.33	45.3	11.28	12.33	17.67	5.23
		0.5	51.03	4.73	4.00	53.3	14.62	16.70	18.00	6.37
		1	51.03	4.43	3.33	50.70	15.81	16.90	21.00	7.93
		1.5	56.00	5.29	3.67	57.30	15.93	17.27	23.33	8.27
LSD 0.05			4.88	0.94	.117	8.17	1.20	1.52	2.37	1.28

Table 1. Effect of citrus rootstocks, Nano NPK fertilizerand their interaction in vegetative and rootgrowth.

Treatment			Total Chlorophyll (SPAD)	Carbohydrates %	N %	P %	K %
Rootstocks	Rangpur		61.67	8.25	2.02	0.16	1.38
	Cleopatra mandarin		59.14	7.82	1.83	0.19	1.31
LSD 0.05			2.34	0.18	0.016	0.013	0.019
	Conc.						
nano NPK Fertilizer ml.L^{-1}	0		56.07	7.33	1.59	0.20	1.16
	0.5		57.03	7.61	1.84	0.22	1.38
	1		62.08	8.04	2.09	0.26	1.45
	1.5		66.43	9.18	2.18	0.30	1.40
LSD 0.05			4.15	0.27	0.013	0.010	0.023
Rootstocks X nano NPK Fertilizer ml.L^{-1}	Rangpur	0	56.30	7.54	1.65	0.20	1.21
		0.5	58.43	7.89	1.88	0.23	1.39
		1	62.97	8.19	2.21	0.23	1.45
		1.5	68.97	9.39	2.34	0.29	1.47
	Cleopatra mandarin	0	55.83	7.11	1.53	0.19	1.11
		0.5	55.63	7.33	1.80	0.21	1.36
		1	61.20	7.88	1.96	0.28	1.45
		1.5	63.90	8.97	2.01	0.31	1.33
LSD 0.05			5.87	0.54	0.031	0.022	0.047

Table 2. Effect of citrus rootstock and Nano NPKfertilizer and their interaction in the chemical content of leaves.

leaf tissues to the effective growth centers and thus improving the growth characteristics of seedlings in general[23]. Or the reason is attributed to the role of Nano-fertilizer and its containment of nutrients (N,P,K) in a ready-to-absorb form, important in increasing meristematic activity and building carbon-forming enzymes and plant hormones, which was positively reflected in increasing plant height, stem diameter, Number of side branches, Number of leaves, leaf area, and building chlorophyll, thus increasing the efficiency of the building process. photosynthesis, accumulation of nutrients and carbohydrates,raising the root efficiency in absorbing water and nutrients and storing them inside plant cells[24] and thus the increase in the absorption of mineral elements by the plant[25]. or Probably is due to the role of the elements the mineral NPK nanoparticles where nitrogen works to stimulate cell division and elongation and increase meristematic activity through its participation in the construction of DNA and RNA and the construction of some plant hormones, including IAA, various enzymes and vitamins[26]. it may be due to the role of phosphorous, which works on the formation of organophosphate compounds in plant tissues necessary for the construction of nucleic acids and phospholipids[27]. or the role of potassium, which is the most important cation in plant physiology for its physiological, chemical and vital functions such as cell division and encouragement of meristem tissue growth[28]. may be due to collectively contribute Nano elements to increasing vegetative growth and absorption of mineral elements and an increase in metabolic reactions, which reflected in the Increase of the Chemical content in the Leaves[29].

Conclusions

The study found that the use of citrus rootstocks and nanofertilizers had a significant positive effect on the growth and chemical content of citrus seedlings. The authors concluded that the observed effects may be due to the following factors:

The different genetic structures of the citrus rootstocks may affect their ability to absorb water and nutrients from the soil and transfer them to the leaves, which can improve the osmotic pressure of cells and photosynthesis.

The small size and high surface area of nanofertilizers may facilitate their penetration into leaf tissues and access to effective growth centers.

Nanofertilizers may contain nutrients in a form that is readily absorbed by plants, which can stimulate meristematic activity, build carbon-forming enzymes and plant hormones, and increase the efficiency of photosynthesis.

The elements nitrogen, phosphorus, and potassium in nanofertilizers may play important roles in cell division, elongation, meristematic activity, nucleic acid synthesis, phospholipid synthesis, and plant growth.

Collectively, the nanoelements in nanofertilizers may increase vegetative growth, absorption of mineral elements, and metabolic reactions, which can lead to increased chemical content in leaves.

The authors' findings suggest that the use of citrus rootstocks and nanofertilizers could be a promising strategy for improving the growth and quality of citrus seedlings. However, further research is needed to confirm these findings and to optimize the use of these technologies in citrus production systems.

According to the outcomes of this study, Rangpur rootstock could be used for grafting because of its appropriate quality characteristics; moreover, its compatibility with all varieties and resistance to viral and fungal diseases. Foliar spraying with Nano NPK fertilizerat a concentration of 150 mg.$^{-1}$.L could be applied to produce strong seedlings.

Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Author Contributions

Methodology and project administration, Harith Al-Tamimi; formal analysis Sabreen Lateef.;validation and writing, Ola Mahmood.

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Conflicts of Interest

The authors declare no conflict of interest.

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