Impact of spraying with Cycocel growth regulator and seaweed extract in growth indicators for local oranges *Citrus sinensis* (L.)

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Abstract

The carried experiment the nurseries located in the Al-Issa area and the wooden canopy / Kufa district in Al-Najaf Al-Ashraf governorate for the agricultural season 2022 to show the effect of spraying with the growth regulator Cycocel concentration (0, 1500, 11000, 11500) mg L^{-1} and spraying with seaweed extract (Super Fifty) At a concentration of (0, 0.25, 0.50, 0.75) ml L^{-1} and their interactions in the growth and vegetative characteristics of one-year-old Local orange seedlings grafted on orange rootstocks. The experiment was carried out Using (RCBD) according to the complete randomized block design, with 3 replications and (16) plants for each replicate. The individual results of two study factors and the interaction between them showed Positive superiority in seedling height, number of leaves, leaf area, the number of branches, and Leaf chlorophyll concentration total. Carbohydrate content in leaves in compared to the comparison (Control) treatment, as for stem diameter, it was not significant in all characteristics of both searches in a manner individually and intertwined.

Keywords: *Citrus sinensis*, Cycocel, Seaweed extracts, growth regulator.

Introduction

The orange, *Citrus sinensis* (L.) Osbeck, belongs to the family Rutaceae, which belongs to the genus Citrus, which includes four economically important acidic groups (the orange group, the bitter orange, the lanky, and the Grapefruit group [1], the regions Tropical and subtropical stretching from Southeast Asia to Indochina are considered the original home of oranges , and citrus fruits rank second in fruit production after grapes in the year, as oranges rank first in citrus production, accounting for 70% of the total production of citrus fruits [2] .

The process of preparing well-grafted seedlings is one of the most important ways of its success, and that the slow growth of citrus seedlings grafted on different rootstocks and the time period it takes to reach the stage suitable for seedling in a permanent place, It is one of the most important problems that leads to an increase in the costs of produc-

ing seedlings, which leads to the application of methods and means to quicken the arrival of the seedlings to the appropriate size, including spraying the vegetative system with plant growth regulators, which have an important and significant role in many important physiological activities within the plant and the regulation of plant growth [3, 4]. Among these regulator is Cycocel. Cycocel is considered a derivative of CCC (chlorocholin chlori) [5] and one of the plant growth retardants,It is used as a synthetic compound that has an anti-gibberellin effect. And it works to hinder and prevent the synthesis of gibberellins due to its interference with the oxidation process by its association with iron for cytochrome P450, thus stopping the production of ent-Kaurenoic acid, which is responsible for cell division and elongation [6]. This leads to a decrease in plant height and an increase in stem diameter, number of branches and leaves, and leaf NPK content [7,8], It has several trade names, such as chloride, Chlormequat, chlorocholine, and CCC.

Seaweed extracts (Super Fifty) are among the organic sources used in agricultural production and are a supplement to fertilizers, not a substitute for them. These extracts boost activities and metabolic processes in areas where they are used. They are antioxidants, root stimulants, flowering agents, and nodes in an effective and safe manner. They also increase the size and symmetry of the fruits. They are resistant to stress and revitalize the plant because they contain many macro nutrients, microelements, amino and organic acids, trace elements, sugars, carbohydrates and growth regulators that work to increase metabolic and structural processes [9].

In an integrated natural form, it significantly drives all the physiological processes of the plant [10,11]. The research aims to study the effect of spraying with Cycocel growth regulator and seaweed extract (Super Fifty) and the interaction between them on local orange seedlings grafted on the *Citrus aurantium* to improving the growth processes, increasing the growth strength of the seedlings, reaching the appropriate size for sale, and determining the best concentration of the materials used.

Materials and Methods

The research was conducted under the wooden canopy of one of the nurseries in the Najaf Al-Ashraf/Kufa Al-Issa region during the growing season 2022–2023. Local orange seedlings, one-year-old, grafted on the Sour Orange *Citrus aurantium*, were selected to be as homogeneous as possible in their growth, as the seedlings were planted in Clay mix soil in 3 Kg plastic sachets. All service operations were carried out, including irrigation, fertilization, crab removal plants, and control of bushes and insects when needed. This experiment was carried out using two factors. The experiment included 48 treatments, which are the interactions between the two experimental factors,the first factor included spraying the shoots with a solution of the cyclocel growth regulator at four concentrations (0, 500, 1000, and 1500) mg L^{-1}, and their symbols are (C3, C2, C1, C0), respectively(Irish origin, highly concentrated up to 65%).As for the second factor, it included 4 concentrations of seaweed extract (Super Fifty) with concentration of (0, 0.25, 0.50 and 0.75) ml L^{-1} with symbols (SF3, SF2, SF1, SF0), respectively, sprayed

on the shoots, and a spreading substance was used (Tween 20) at a concentration of (0.1) ml L^{-1} to the spray solution , natural extract contains 100% Ascophyllum nodosum, it contains all that the plant needs in major and minor nutrients, in addition to amino and organic acids, growth regulators, trace elements, sugars, and carbohydrates, all in an integrated natural form that significantly drives all the physiological processes of the plant. An antioxidant that stimulates the roots, pushes flowering and knots effectively and safely, increases the size and uniformity of fruits, resists stress, and revitalizes plants.

The study was carried out as a factorial experiment with two factors (4 x 4) according to the complete randomized block design (RCBD) with three replications. Averages were compared using the least significant difference test (L.S.D.) at a probability level of 0.05 [12].

Studied traits:

1. Seedling height (cm): It was measured from the site of contact with the soil to the end of the growing top of the plant.
2. The diameter of the main stem (mm) : It was measured by the foot (Vernia Caliper) from the area of contact of the fifth true leaf with the stem.
3. Number of leaves: The number of full-grown leaves on the plant was calculated.
4. Leaf area (cm^2): Three full-width leaves were taken from each plant in the experimental unit and measured using the method used by [13]using a scanner with a program (ImageJ) and the rate was taken to calculate the leaf area.
5. Number of branches (shoot $seedlings^{-1}$): The number of new growths of seedlings was calculated.
6. The percentage of carbohydrates in the diet: This characteristic was estimated by the acid colormetric method (PSACM) Modification of phenol sulphric mentioned by [14].
7. Leaves content of total chlorophyll (mg $100g^{-1}$ fresh weight): It was estimated according to the method [15].

Result and Discussion

Seedling height (cm)

The results in Table (1) show that there was a significant effect of spraying with seaweed extract (Super Fifty), as the concentration exceeds (0.75 ml L^{-1}) over the rest, giving the highest height of the seedling length, as it reached (124.7 cm) compared to the lowest rate of seedling height (115.4 cm) with the control treatment. As for the spraying with the Cycocel growth regulator, the concentration (1500 mg L^{-1}) had a significant effect on the decrease in the height of the seedlings, reaching 117.5 cm compared to the control treatment. As for the interaction between the seaweed extract (Super Fifty) and the growth regulator cycocel, there was a significant difference between the treatments, as the treatment excelled (0 + 0.75) by obtaining the highest rate of seedling

length, which reached (131.02 cm) compared to the lowest rates (110.04 cm) in the comparison treatment.

Table (1): Effect of spraying with growth regulator cycocel and seaweed extract and the interaction between them on seedling height (cm)

concentration Cycocel mg L^{-1}	Seaweed extract concentrations (Super Fifty) mg L^{-1}				Cycocel average
	0	0.25	0.5	0.75	
0	110.0	118.3	127.4	131.0	121.7
500	117.8	121.1	127.7	128.6	123.8
1000	118.0	119.5	121.3	120.5	119.8
1500	116.0	116.9	118.5	118.6	117.5
average seaweed extract	115.4	118.9	123.7	124.7	
L.S.D	concentration Cycocel		concentration seaweed extract		Interaction
0.05	0.0072		0.0072		0.0144

Stem diameter (mm)

The results in Table 2 showed that spraying with Cycocel growth regulator and seaweed extract (Super Fifty) individually and interaction between them gave an increase in the average of stem diameter, but it did not reach significance.

Table (2): Effect of spraying with growth regulator Cycocel and seaweed extract and the interaction between them in stem diameter (mm)

concentration Cycocel mg L^{-1}	Seaweed extract concentrations (Super Fifty) mg L^{-1}				Cycocel average
	0	**0.25**	**0.5**	**0.75**	
0	2.31	2.34	2.38	2.42	2.36
500	2.24	2.27	2.3	2.32	2.28
1000	2.22	2.17	2.18	2.2	2.19
1500	2.19	2.12	2.14	2.17	2.16
average seaweed extract	2.24	2.23	2.25	2.28	
L.S.D	concentration Cycocel		concentration seaweed extract		Interaction
0.05	ns		ns		ns

Number of leaves (leaves seedlings^{-1})

The results of Table (3) indicate that there are significant differences in spraying with the growth regulator Cycocel, where the treatment with a concentration of (1500) mg

L^{-1} excelled, as it gave the highest average number of leaves, which reached (138.59 leaves seedlings^{-1}). It is also noted from Same table that spraying with seaweed extract had a significant effect on the average number of leaves, as it reached the highest rate when treated with a concentration of (0.50 mg L^{-1}), reaching (147.22 leaves seedlings^{-1}), while the Control treatment gave the lowest rate in the number of leaves. (138.19 leaves seedlings^{-1}).

The interaction effect of the two study factors spraying with the growth regulator Cycocel and seaweed extract was significant, as the interaction treatment excelled at a concentration of (500 + 0.50) mg L^{-1} by giving it the highest rate in the number of leaves, reaching (158.68 leaves seedlings^{-1}) compared to the Control treatment of (131.04 leaves seedlings^{-1}).

Table (3): The effect of spraying with the growth regulator Cycocel and seaweed extract and the interaction between them on the number of leaves

concentration Cycocel mg L^{-1}	Seaweed extract concentrations (Super Fifty) mg L^{-1}				Cycocel average
	0	0.25	0.5	0.75	
0	131.04	139.96	148.40	157.02	144.10
500	145.77	148.76	158.68	149.63	150.71
1000	138.98	140.47	142.34	141.48	140.82
1500	136.99	137.91	139.48	138.59	138.24
average seaweed extract	138.19	141.77	147.22	146.68	
L.S.D	concentration Cy-cocel		concentration sea-weed extract		Interaction
0.05	1.876		1.876		3.751

Leaves area (cm^2)

The results in Table 4 showed that spraying with Cycocel growth regulator gave significant differences in leaf area, where the treatment with a concentration of (500 mg L^{-1}) excelled, reaching (300.9 cm^2) compared to other concentrations and the Control treatment.

It is noted from the same table that spraying seaweed extract had a significant effect on the mean leaf area, as it reached the highest rate when treated with a concentration of (0.50 ml L^{-1}), reaching (147.22 cm^2), while the Control treatment gave the lowest rate in leaf area (147.22 cm^2).

The interaction effect of the two study factors spraying with growth regulator cycocel and seaweed extract was significant, as the treatment of Interaction ping with a concentration of (500 + 0.50) excelled by giving it the highest rate of leaf area, which amounted to (309.10 cm^2) compared to the Control treatment of (281.23 cm^2).

Table (4): The effect of spraying with the growth regulator Cycocel and seaweed extract and the interaction between them on leaf area (cm2)

concentration Cycocel mg L^{-1}	Seaweed extract concentrations (Super Fifty) mg L^{-1}				Cycocel average
	0	0.25	0.5	0.75	
0	281.2	290.1	298.3	307.4	294.2
500	296.1	299.1	309.1	299.6	300.9
1000	288.9	290.4	292.3	291.4	290.8
1500	286.9	287.9	289.4	288.5	288.2
average seaweed extract	288.3	291.9	297.3	296.7	
L.S.D	concentration Cycocel		concentration seaweed extract		Interaction
0.05	1.889		1.889		3.778

Number of shoots (shoot seedlings^{-1})

It is noted from the data in Table (5) that spraying with the growth regulator Cycocel had a significant effect on increasing the number of branches, as the highest value was recorded at a concentration of (1500 mg L-1), as it yielded (5.81 shoot seedlings^{-1}), with the Control treatment, which had a value of (4.92 shoot seedlings^{-1}) .

The results of Table 5 indicate that spraying with seaweed extract (Super Fifty) indicated that there were significant differences in the number of branches with its different concentrations, as it yielded the highest values rate when treated with a concentration of (0.50 ml L^{-1}), reaching (6.40 shoot seedlings^{-1}), while the Control treatment reached the lowest number of branches with a value of (4.49 shoot seedlings^{-1}).

As for the interaction between the two factors spraying with the growth regulator Cycocel and seaweed extract (Super Fifty) as in the data of Table No. (5), the treatment of interaction excelled at a concentration of (1500 + 0.75) by giving the highest significant increase in the number of branches, reaching (6.57 shoot seedlings^{-1}), which in turn outperformed the rest of the treatments and the Control treatment with a value of (3.55 shoot seedlings^{-1}).

Table (5): Effect of spraying with growth regulator cycocel and seaweed extract and the interaction between them on the number of branches (shoot seedlings^{-1})

concentration Cycocel mg L^{-1}	Seaweed extract concentrations (Super Fifty) mg L^{-1}				Cycocel average
	0	0.25	0.5	0.75	
0	3.55	4.30	5.55	6.30	4.92
500	4.71	5.33	5.67	6.43	5.53
1000	4.81	5.37	5.77	6.50	5.61
1500	4.91	5.43	6.33	6.57	5.81
average seaweed extract	4.49	5.11	5.53	6.40	
L.S.D	concentration Cycocel		concentration seaweed extract		Interaction
0.05	0.2466		0.2466		0.4933

leaf content of total chlorophyll (mg 100 g^{-1} fresh weight)

The effect of search treatments on the leaves content of total chlorophyll, as the results showed in Table (6) significant differences by spraying with the growth regulator Cycocel, as the treatment with a concentration of (1500 mg L^{-1}) excelled by giving it the highest value of (281.4 mg 100 g^{-1} fresh weight) While the lowest content of total chlorophyll in the leaves was (243.3 mg 100 g^{-1} fresh weight) in control treatment.
The data of the same table showed that spraying with seaweed extract (Super Fifty) indicated a significant difference in the total chlorophyll content of the leaves, as the concentration (0.75 mg L^{-1}) reached the highest rate (307.2 mg 100 g^{-1} fresh weight), while it gave The Control treatment had the lowest significant difference (224.3 mg 100 g^{-1} fresh weight). The two research factor sprayed with the growth regulator Cycocel and seaweed extract (Super Fifty) in the total chlorophyll content of the leaves showed an interaction, the treatment (1000 + 0.75) mg L^{-1} excelled by giving it the highest value as it reached (311.2 mg 100 g^{-1} fresh weight) with the Control treatment that gave The lowest values if it reached (177.1 mg 100 g^{-1} fresh weight).

Table (6): Effect of spraying with growth regulator cycocel and seaweed extract and the interaction between them on the leaves' total chlorophyll content (*mg 100 g^{-1}* fresh weight)

concentration Cycocel mg L^{-1}	Seaweed extract concentrations (Super Fifty) mg L^{-1}				Cycocel average
	0	0.25	0.5	0.75	
0	177.1	214.7	276.9	304.6	243.3
500	235	266.1	282.9	309.4	273.4

1000	240	268.1	287.9	311.2	276.8
1500	245	271.1	306.1	303.6	281.4
average seaweed extract	224.3	255	288.5	307.2	
L.S.D	concentration Cy-cocel		concentration seaweed extract		Interaction
0.05	9.97		9.97		19.94

Percentage of total carbohydrates in leaves (%)

It can be seen from the results of Table (7) that the effect of spraying with the growth regulator Cycocel was significant in the percentage of total carbohydrates in the leaves at a concentration of (1500 mg L^{-1}), which achieved the highest significant value of (21.54%), while the Control treatment gave the least significant difference, as (20.65%).

Same table shows that there is a significant difference when spraying with seaweed extract (Super Fifty), where the concentration exceeds (0.75 ml L^{-1}) in increasing the percentage of total carbohydrates in the leaves, as it amounted to (22.18%), compared with the Control treatment, which amounted to (20.22 %).

The interaction of the research factors, spraying with the growth regulator Cycocel and seaweed extract (Super Fifty), had a significant effect in increase the percentage of total carbohydrates in the leaves, where the concentrations of interaction exceeded (1500 + 0.75) and (1000 + 0.5) mg L^{-1} in this capacity, reaching (22.30%). and (22.23%), respectively, compared to what was achieved by the Control treatment, with the lowest rate of (19.28%).

Table (7): Effect of spraying with growth regulator Cycocel and seaweed extract and the interaction between them on the percentage of total carbohydrates in the leaves (%)

concentration Cycocel mg L^{-1}	**Seaweed extract concentrations (Super Fifty) mg L^{-1}**				**Cycocel average**
	0	0.25	0.5	0.75	
0	19.28	20.03	21.28	22.03	20.65
500	20.44	21.06	21.4	22.16	21.26
1000	20.54	21.1	21.4	22.23	21.34
1500	20.64	21.16	22.06	22.30	21.54
average seaweed extract	20.22	20.84	21.56	22.18	
L.S.D	Concentration Cycocel		concentration seaweed extract		Interac-tion
0.05	0.25		0.25		0.50

Table (1) shows that the growth regulator harms the seedling height by giving the lowest rate compared to the control treatment. The reason may be attributed to the

growth regulator Cycocel working to reduce stem elongation by inhibiting cell division in the sub-apical meristem without affecting the apical meristem, hence decreasing plant growth and elongation [16,17], this is similar with what [18] discovered when multiple amounts of the Cycocel growth regulator were applied to the larch plant. This is consistent with what was reached [19]. when multiple amounts of the Cycocel growth regulator were applied on the Cardinia plant [20].

The interaction of the study factors led to an increase in plant height due to the addition of the seaweed extract (Super Fifty) containing amino acids, which are natural compounds that help the balanced growth of the plant and strengthen it, increase chlorophyll, and reduce stress. This is attributed to the presence of active auxin, gibberellin, and vitamins in the seaweed extract, and these substances helped activate cytokinin, which in turn prevented oxidation. It contains a substance that prevents the oxidation of chlorophyll, in addition to the presence of magnesium, and it has a direct role in building the chlorophyll molecule [21]. While we did not notice any significant effect of the experimental factors on the average number of leaves.

Tables (3, 4, 5, 6, and 7) showed that the studied traits qualities (number of leaves, leaf area, number of branches, chlorophyll content, and carbohydrate content) ,the reason is attributed to Cycocel's role in its anti-gibberellin effect by inhibiting the production of (ent-Kaurenoic acid), which is responsible for cell division and elongation [22], resulting in a decrease in plant height and an increase in stem diameter, number of branches and leaves, and leaf NPK content [7,23], it encourages lateral branching, or perhaps reduces the level of internal auxins in seedlings, especially the growth promoter (IAA), which is considered one of the most important factors causing apical dominance in plants, leading to the formation of additional branches [24,25], by increasing the plant content of cytokinins [26], thus, an increase in the building of chlorophyll or preventing its degradation. The reason for the increase in the percentage of carbohydrates may be attributed to the spraying of the growth regulator Cycocel and seaweed extract, whose seedlings gave the largest percentage in leaf area and the highest percentage of chlorophyll pigment, and this led to an increase in the manufacture of nutrients in the leaves, which led to a high percentage of carbohydrates. These results agree with [27] when spraying a hybrid (Lilium spp. L.) and [28] , when spraying ornamental plants, and [29] when spraying okra.

We also note that spraying seaweed extract has a positive role in increasing growth indicators as a result of what it contains in terms of growth stimuli, security, and organic acids, which have an effective and important role in vegetative growth and the emergence of the chlorophyll molecule, which is the basis of the photosynthesis process, and increases its outputs in the leaves and provides the energy necessary for growth and construction [30]. It also contains many major and minor natural elements and amino acids, which play an effective role in increasing vegetative growth [31,32], there is also an increase in auxin-like substances in seedlings treated with seaweed extract [33], as well as the content of marine algae extract of essential elements for growth such as nitrogen, which has an effective and important role and a wide range in

the vital activity of plants, and increased uptake by the plant, which reflected positively on the increase in vegetative growth [34],these results are in agreement with [35] When spraying seaweed extract on peach seed germination and seedling growth (*Prunus persica* L.), [36] when spraying *Freesia hybrida* L. and [37] when spraying local apricot seedlings, and with the results of [38] when spraying olive seedlings.

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