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Effect of CuSO4 and seaweed extract on the nutritional status of grafted local lemon seedlings Citrus limon L. on aurantifolia

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***Abstract*---**Citrus aurantifolia rootstock seedlings were treated with seaweed extract and copper sulfate. Seaweed extract (ground feed) was added during the growing season at four levels (0, 10, 20, 30 ml seedlings^{-1}) and copper sulfate sprayed at four concentrations (0,5, 10, 15 ml L^{-1}). The results showed that the treatment of lemongrass seedlings grafted on rootstock aurantifolia with seaweed extract and spraying with copper sulfate affected the nutritional status For seedlings (percentage of nitrogen in shoots (%), percentage of phosphorous in shoots (%), percentage of potassium in shoots (%), content of leaves of copper (mg. kg-1), iron content of leaves (mg The results also showed that the addition of marine algae extract at a concentration of 30 ml interfering with spraying with copper sulfate at a concentration of 15 ml L^{-1} is recommended to improve the vegetative, root and nutritional growth of lemon seedlings and its content of macro and micro nutrients.

***Keywords*---**citrus aurantifolia, seaweed extract, copper sulfate CuSO4.

Introduction

Citrus belongs to the family Rutaceae, which grows in the tropics and subtropics, and the regions of Southeast Asia are the rootstock for it. In Iraq, cultivation of most types of citrus is spread in the central and southern regions. And the environment, as its fruits are rich in salt, sodium, sulfur and phosphorous. It is also a source of vitamin C and good amounts of vitamins A, B1 and B2 (Dwai and

Fadila, 2016 and Ibrahim, 2015). Lemon (Citrus limon L.) Burm is one of the citrus species of the genus Citrus The regions of northeastern India and southwestern China are the habitat of rootstock for it (Ismail and Zhang, 2004), and most citrus species, including local lemon, are propagated on different citrus roots, including aurantifolia. Several studies have indicated a mutual effect between rootstock and scion in citrus fruits. The effect of rootstock in scion was more pronounced (Agusti, 2003). Seaweed extracts are among the The important organic sources used in agricultural production are complementary to, and not a substitute for, fertilizers, as they stimulate the physiological functions of the plant because it contains many elements as it contains major nutrients N, P, K and micro nutrients such as Fe, B, Zn as well as growth-promoting substances Such as auxins, gibberellins, and cytokinins. It also contains some amino and organic acids, which are necessary for building protein (Hegab et al., 2005) and have great physiological effects when added to the soil, as it increases plant resistance to unsuitable conditions or infection with diseases and insects. These extracts are used about fifteen million tons. annually in various countries of the world (Spinilli et al., 2009). It also leads to stimulating root growth, increasing stem thickness, and increasing vegetative growth by increasing the efficiency of the photosynthesis process, in addition to protecting the plant from stress conditions such as drought, salinity and aging by supporting the plant cell) Al-Hayani et al. (2014). Microelements also play an important role in the natural growth of the plant and in the completion of Its life cycle and plants need such elements in very small quantities despite their importance and their involvement in physiological processes and enzymatic reactions (Abu Dhahi et al., 1988). 2000) Copper also participates in the vital processes of the formation of proteins and carbohydrates and plays a role in the photosynthesis process through its role in the chlorophyll formation process, as a high percentage of total copper is found in the chloroplasts. Electronic transport chain linking the two photochemical reaction systems of photosynthesis (Mahler) 200 4). Based on the above and the lack of studies on it, the study aimed at the following:

1. Studying the effect of seaweed extract and copper and the interaction between them on vegetative and root growth characteristics and nutritional content in order to obtain strong seedlings of local lime.
2. Studying the effect of rootstock used in grafting on the growth and nutritional status of growing local lemongrass seedlings on rootstock aurantifolia as a result of the deterioration of citrus cultivation in Iraq, with the aim of increasing the seedling growth rate and transporting to the permanent place in the shortest possible period and increasing its size through foliar feeding and ground feeding.

Materials and Methods

The study was carried out in a certified citrus production nursery in the district of Al-Hindiya - the holy governorate of Karbala, which belongs to the Iraqi Ministry of Agriculture - General Directorate of Horticulture and Forests for the period from 15/3/2020 to 10/15/2021 to study the effect of ground fertilization with seaweed extract Algaton and copper sulfate $CuSO_4$ (6.9% copper). In the growth of lemongrass seedlings grafted on rootstock and aurantifolia. 384 seedlings of homogeneous growth as possible were selected from Citrus limon L. (Burm) seedlings of six months old, with a height of (30-35) cm, each seedling was planted in a plastic pot, 23 cm high, with a diameter of 24 cm from the top and 18 cm from the bottom. Capacity of 10 kg of soil, the same size as possible from the planting beds in the nursery for the production of certified citrus seedlings, and each seedling was planted in a plastic pot of 10 kg of soil. Container of a mixture of river mixture and peat moss (2 volumes: 1 volume) and placed in the wire canopy covered with saran for the duration of the experiment. The service operations were carried out equally for all transactions, from watering the seedlings by spraying and sometimes manually with equal quantities of irrigation water, removing the bush and controlling the process of all plants periodically. Samples were taken from the agricultural medium in which the seedlings were growing, then mixed in a homogeneous manner, then analyzed in the College of Agriculture - University of Kufa to know some of its physical and chemical properties in the laboratories of the Soil and Water Department according to the methods mentioned in Page et al. (1982) and the results of the analysis are shown in Table (1).

Experience design

The treatments in the experiment were distributed in the system of Split-Split Plot Design with three replications in the design of randomized complete sectors (R.C.B.D). The main-plots represented four levels (30,20,10,0 ml seedling^{-1}) of ground fertilization with seaweed extract containing Some major elements in addition to the organic matter (Table No. 2) The addition of a floor to each anvil as an injection into the soil of the anvil in the extension area of the seedling roots with three additions and a period of 30 days between one addition and another. The sub-plots are four levels (0,5,10,15 mg L^{-1}) From the foliar feeding with copper element in the form of copper sulfate by spraying on the vegetative total of the leaves until the complete wetness of the seedlings with three additions and a period of 30 days between one addition and another. And the assets in the sub-sub-plots panels, as 384 seedlings of homogeneous growth and age were selected and divided into 32 treatments, and each treatment was repeated three times, with 5 seedlings for one replicate (4 x 4 x 2), with 3 replications and 5 seedlings for each treatment in each replicate Each repeat contains 128 seedlings. (Al-Rawi and Khalaf Allah, 1980). Spraying treatments were carried out at a rate of 6 sprays (three in the spring season and the same in the fall season). The first spray was carried out on 3/15/2020, the second spray was on 4/4/2020 and the third spray was on 15/5/2020 after which the spraying was stopped Until the middle of the eighth month of high temperatures and 3 sprays were conducted on 15/8/2020 and 15/9/2020 and the last spray on 10/15/2020, as the ground for plants was added with seaweed extract in the early morning and the plants were sprayed with copper sulfate three days after it. Whereas, the comparison treatment was sprayed with distilled water only, and a 2-liter hand sprayer was used. The plants were watered abundantly a day before the spraying date until complete wetness to increase the efficiency of the plants in absorbing the sprayed substance, as moisture plays a role in the process of swelling the guard cells and opening stomata as well as The fact that watering before spraying reduces the concentration of solutes in the leaf cells, which increases the penetration of the ions of the spray solution into the leaf cells. A suitable distance was left between treatments and nylon barriers were used to avoid the effect of the volatile spray between treatments. Drops of liquid soap were added to the spray solutions as a diffuser.

Table 2: The components of seaweed extract with some nutrients used in the experiment, produced by Artal Company (Valencia - Spain)

Component	%
N	4%
P	4 %
K	4%
Mg	32ppm
Fe	30ppm
Cu	14.5ppm
Mn	31ppm
Zn	17ppm
Auxin-likembiological activity	13.mg
Cytokinin-like biological activity	0.2mg
Protein	5.0 g
Glutamic Acid	36 mg
Ascorbic acid	17.6 mg
Glycine	70 mg
Fulvic acid	0.25%
Organic matter	12%

The following characteristics were measured: The percentage of nitrogen in the shoot (%), the percentage of phosphorous in the shoot (%), the percentage of potassium in the shoot (%), the content of leaves of copper (mg. kg -1), the content of leaves of iron (mg. kg -1).

Results

Percentage of nitrogen in the shoot (%)

The results in Table (3) showed that the fertilizing treatment with seaweed extract at a concentration of 30 ml significantly affected the nitrogen content of leaves, which amounted to 1.88% and 2.40% for the two seasons, respectively, compared to the plants that were not fertilized, which recorded the lowest nitrogen content in leaves 1.48% and 1.70% for both seasons. Experiment sequentially, as it appeared from the results of the same table that there was a significant effect on the percentage of plant nitrogen, and the highest values were achieved after spraying with a concentration of 15 ml. L-1 $CuSo_4$ copper sulfate amounted to 1.75% and 2.16% for the two seasons of the experiment, respectively, compared to the comparison treatment, which gave the lowest values of 1.63% and 1.93% for the two seasons, respectively. .16% for the two seasons of the experiment sequentially, as the results of the same table showed that the interaction between the fertilization with seaweed extract and the roots had a significant effect in increasing the percentage of leaves from nitrogen, as the treatment of 30 ml × rootstock aurantifolia gave the highest percentages, amounting to 2.01% and

2.72% for the two seasons of the experiment sequentially. Significant effect of the interaction between the roots and copper sulfate treatment, where the rootstock aurantifolia was significantly superior with 15 mg L-1 CuSo4 and gave the highest percentages of nitrogen, which amounted to 1.87% and 2.37% for the two seasons of the experiment respectively. Treatment of 30 ml seaweed extract x 15 mg L-1 CuSo4 achieved the highest percentages of 1.96% and 2.88% compared to the lowest nitrogen content when treating 0 ml seaweed extract x 0 mg L-1 CuSo4 which was 1.35% and 1.50% for the two seasons of the experiment, respectively.

Table1. Effect of fertilization with seaweed extract and copper sulfate and the interaction between them on the percentage of nitrogen (%) of rootstock aurantifolia

	Nitrogen %									
	First season					Second season				
Algae Extract . (ml.seedling^{-1})	$CuSO_4$ (mgL^{-1})				Average	$CuSO_4$ (mg.L^{-1})				Average
	0	5	10	15		0	5	10	15	
0	1.47	1.55	1.68	1.63	1.58	1.69	1.83	1.96	1.82	1.83
10	1.72	1.77	1.80	1.81	1.77	1.94	1.78	1.99	2.03	1.93
20	1.85	1.89	1.91	1.92	1.89	2.34	2.03	2.11	2.22	2.18
30	1.94	1.97	1.99	2.14	2.01	2.65	2.33	2.48	3.40	2.72
Average	1.74	1.79	1.84	1.87		2.15	1.99	2.14	2.37	2.16
L.S.D. (P≤0.05)	Algae Extract	$CuSO_4$	Inter.			Algae Extract	$CuSO_4$	Inter.		
	0.08	0.00	0.02			0.01	0.07	0.18		

Percentage of phosphorous in the shoot (%)

It was found from Table (5) that the experimental treatments had a significant effect on the percentage of phosphorous, and the moral superiority of fertilizing with seaweed extract at a concentration of 30 ml was noted, which recorded the highest averages of 0.30 and 0.33% for the two seasons, respectively, compared to plants that were not fertilized, which recorded the lowest content of phosphorous in leaves. 18% and 0.19% for the two seasons of the study respectively, and it was noticed that there was a significant superiority of the plants sprayed with the concentration of 15 ml. L-1 CuSo4 copper sulfate amounted to 0.26% and 0.29% for the two seasons of the experiment, respectively, compared to the control treatment, which gave the lowest values, which were 0.22% and 0.24% for the two seasons, respectively. As for rootstock, it significantly affected the percentage of phosphorous, as the aurantifolia rootstock outperformed and achieved the highest averages of 0. 27 % and 0.29% for the two seasons of the experiment sequentially, as the results of the same table showed that the interaction between the soil fertilization with seaweed extract and rootstock had a significant effect, and the treatment of 30 ml × rootstock aurantifolia achieved the highest percentages, which amounted to 0.35% and 0.38% for the two seasons of the experiment sequentially, and the results showed a significant effect of the treatment The interaction between the roots and copper sulfate, where it significantly outperformed rootstock aurantifolia with 15 mg.L-1 CuSo4 and gave the highest percentages, which were 0.30% and 0.32% for the two seasons of the experiment, respectively.

Table2. Effect of fertilization with seaweed extract and copper sulfate and the interaction between them on the percentage of phosphorous (%) of rootstock aurantifolia

	Phosphorous									
	First season					Second season				
Algae Extract . (ml.seedling^{-1})	$CuSO_4$ (mgL^{-1})				Average	$CuSO_4$ ($mg.L^{-1}$)				Average
	0	5	10	15		0	5	10	15	
0	1.25	1.39	1.41	1.50	1.39	1.25	1.38	1.40	1.48	1.38
10	1.51	1.56	1.63	1.62	1.58	1.50	1.57	1.64	1.59	1.57
20	1.67	1.74	1.82	1.87	1.77	1.75	1.73	1.81	1.86	1.79
30	1.95	1.97	2.12	2.36	2.10	2.14	1.95	2.15	2.68	2.23
Average	1.59	1.66	1.74	1.84		1.66	1.66	1.75	1.90	
L.S.D. ($P \leq 0.05$)	Algae Extract		$CuSO_4$	Inter.		Algae Extract		$CuSO_4$	Inter.	
	0.00		0.00	0.00		0.00		0.00	0.15	

Percentage of potassium (%)

It is noted from the results contained in Table (3) that the experimental treatments and all their interactions had a significant effect on the percentage of potassium. It was noted that there was a significant superiority in the plants treated with seaweed extract at a concentration of 30 ml, which amounted to 1.95% and 2.03% for the two seasons, respectively, compared to the plants that were not fertilized and which recorded The lowest percentage of potassium in leaves was 1.28% and 1.30% for the two seasons of the experiment respectively, as it was shown through the same results that there was a significant effect in the percentage of potassium in the leaves of the plant, and the highest values were achieved after spraying with a concentration of 15 ml. L-1 CuSo4 copper sulfate amounted to 1.71% and 1.72% for the two seasons of the experiment, respectively, compared to the control treatment, which gave the lowest values, which were 1.49% and 1.56% for the two seasons, respectively. .74% for the two seasons of the experiment sequentially, as the results showed in the same table that the interaction between fertilization with seaweed extract and rootstock had a significant effect in increasing the potassium content of leaves, as the treatment of 30 ml × rootstock aurantifolia gave the highest percentages, amounting to 2.10% and 2.23% for the two seasons of the experiment sequentially compared to By the non-fertilization treatment with Citrus rootstock, which gave the lowest values were 1.19% and 1.22% for the two seasons of the experiment sequentially, the results showed that there was a significant effect of the interaction treatment between the roots and copper sulfate, where it significantly outperformed rootstock aurantifolia with 15 mg L-1 CuSo4 and gave the highest percentages of potassium amounted to 1. 71% and 1.74% for the two seasons of the experiment, respectively.

Table3. Effect of fertilization with seaweed extract and copper sulfate and the interaction between them on the percentage of potassium (%) of aurantifolia rootstock

	K %									
Algae Extract . (ml.seedling^{-1})	First season					Second season				
	$CuSO_4$ (mgL^{-1})				Average	$CuSO_4$ (mg.L^{-1})				Average
	0	5	10	15		0	5	10	15	
0	1.25	1.39	1.41	1.50	1.39	1.25	1.38	1.40	1.48	1.38
10	1.51	1.56	1.63	1.62	1.58	1.50	1.57	1.64	1.59	1.57
20	1.67	1.74	1.82	1.87	1.77	1.75	1.73	1.81	1.86	1.79
30	1.95	1.97	2.12	2.36	2.10	2.14	1.95	2.15	2.68	2.23
Average	1.59	1.66	1.74	1.84		1.66	1.66	1.75	1.90	
L.S.D. ($P \leq 0.05$)	Algae Extract	$CuSO_4$	Inter.			Algae Extract	$CuSO_4$	Inter.		
	0.00	0.00	0.00			0.02	0.01	0.05		

Copper percentage (mg.kg-1)

It appears from the results of Table (4) that the experimental treatments and all their interactions had a significant effect on the content of copper in the leaves. It was noted that there was a significant superiority in the plants treated with seaweed extract at a concentration of 150 ml, which amounted to 23.79 and 26.54 mg. kg-1 for the two seasons, respectively, compared to the plants that did not fertilize, which recorded the lowest averages in leaves of 14.74 and 13.77 mg. kg -1 for the two seasons of the experiment sequentially, as it appeared from the results of the same table that there was a significant effect on the copper content of the leaves, and the highest values were achieved after spraying with a concentration of 15 ml. L-1 CuSo4 copper sulfate amounted to 21.39 and 26.54 mg. kg -1 for the two seasons of the experiment sequentially compared to the control treatment, which gave the lowest values of 18.71 and 18.86 mg. kg -1 for the two seasons respectively, and aurantifolia rootstock had a significant effect and the highest averages were 22.70 and 22.12 mg. kg -1 for the two seasons of the experiment sequentially, as the results of the same table showed that the interaction between fertilization with seaweed extract and the roots had a significant effect in increasing the copper content of leaves, as the treatment of 30 ml × rootstock aurantifolia gave the highest values, amounting to 26.43 and 28.49 mg. kg -1 for the two seasons of the experiment sequentially. The results showed a significant effect of the interaction treatment between the roots and copper sulfate, where it significantly outperformed rootstock aurantifolia with 15 mg L-1 CuSo4 and gave the highest percentages of copper amounting to 24.26 and 24.16 mg. kg -1 for the two seasons of the experiment sequentially.

Table4. Effect of fertilization with seaweed extract and copper sulfate and the interaction between them on the percentage of copper (mg.kg-1) for aurantifolia rootstock

	Cu(mg.kg-1)									
Algae Extract ml.seedling^{-1}	First season					Second season				
	$CuSO_4$ (mgL^{-1})				Average	$CuSO_4$ ($mg.L^{-1}$)				Average
	0	5	10	15		0	5	10	15	
0	15.47	16.60	17.55	19.37	17.25	13.77	14.57	16.71	16.92	15.49
10	20.52	21.79	22.91	23.67	22.22	18.95	17.79	19.20	21.54	19.37
20	23.82	24.13	24.96	26.67	24.90	22.82	24.62	25.63	27.48	25.14
30	25.58	26.08	26.72	27.33	26.43	26.95	27.61	28.69	30.72	28.49
Average										
L.S.D. ($P\leq0.05$)	Algae Extract	$CuSO_4$	Inter.			Algae Extract	$CuSO_4$	Inter.		
	0.27	0.19	0.62			0.23	0.16	0.48		

Iron Percentage (mg.kg -1)

It is clear from table (5) that the experiment's treatments and all their interactions significantly affected the iron content of the leaves. The significant superiority was observed in plants treated with seaweed extract at a concentration of 30 ml, which achieved the highest percentages of 84.103 and 93.26 mg. kg -1 for the two seasons, respectively, compared to the plants that did not fertilize, which recorded the lowest averages of 58.66 and 61.60 mg. kg -1 for the two seasons of the experiment sequentially, as it appeared from the results of the same table that there was a significant effect on the iron content of leaves, and the highest values were achieved after spraying with a concentration of 15 ml. L-1 CuSo4 copper sulfate amounted to 74.72 and 82.05 mg. kg-1 for the two seasons of the experiment sequentially compared to the comparison treatment, which gave the lowest values of 67.62 and 74.21 mg. kg -1 for the two seasons respectively, and aurantifolia rootstock had a significant effect, and the highest averages were 76.04 and 81.80 mg. kg -1 for the two seasons of the experiment sequentially, as the results of the same table showed that the interaction between fertilization with seaweed extract and the roots had a significant effect in increasing the iron content of leaves, as the treatment of 30 ml × rootstock aurantifolia gave the highest values, amounting to 90.14 and 97.34 mg. kg -1 for the two seasons of the experiment sequentially, and the results showed a significant effect of the interaction treatment between the roots and copper sulfate, where it significantly outperformed rootstock aurantifolia with 15 mg l-1 copper sulfate, and gave the highest percentages of iron amounting to 78.89 and 86.28 mg. kg -1 for the two seasons of the experiment sequentially.

Table 5 Effect of fertilization with seaweed extract and copper sulfate and the interaction between them on the percentage of iron (mg.kg-1) for aurantifolia rootstock

	Iron Percentage (mg.kg -1)									
Algae Extract. (ml.seedling $^{-1}$)	First season					Second season				
	$CuSO_4$ (mgL^{-1})				Average	$CuSO_4$ ($mg.L^{-1}$)				Average
	0	5	10	15		0	5	10	15	
0	57.54	62.16	63.99	65.91	62.40	60.17	62.36	65.99	69.49	64.51
10	68.05	69.37	70.91	73.97	70.58	70.48	73.52	79.13	82.61	76.44
20	76.66	81.38	82.81	83.39	81.06	85.66	86.51	89.64	93.85	88.92
30	86.43	90.27	91.56	92.28	90.14	99.17	95.74	95.29	99.18	97.34
Average	67.62	70.93	73.09	74.72		74.21	76.26	78.69	82.05	
L.S.D. ($P \leq 0.05$)	Algae Extract	$CuSO_4$	Inter.			Algae Extract	$CuSO_4$	Inter.		
	0.51	0.44	0.96			0.75	0.47	1.28		

Discussion

The reason for the increase in the proportion of nitrogen, phosphorous and potassium elements in Tables (3, 4, and 5) in the vegetative total as a result of the response to seaweed extract is due to the ability of the latter to increase these elements in the soil and provide them in an accessible manner for uptake by the plant (Maleki et al., 2013) or it may be due to the role of What seaweed extract contains in increasing the development of vegetative growths, which was reflected in the formation of a good root system, which led to an increase in the efficiency of absorption of elements, including nitrogen, phosphorous and potassium (Priya, 2013).

The treatments led to the emergence of a significant increase in the ratios of major elements N, P, K, in addition to the minor elements in the above tables, compared to the comparison treatment. The organic acids present in the fertilizer lead to an increase in the permeability of cell membranes and to facilitate the transfer of nutrients, especially N, P and K, which led to an increase in the efficiency of the plant to absorb and accumulate these elements in the leaves (Hashem, 2014). These nutrients are very important for the plant, as nitrogen is included in the composition of the amino acids and the resulting proteins, as adding it leads to the provision of the necessary nutrients in a ready-made way to the soil, and the roots of seedlings can absorb these nutrients and benefit from them, especially the major elements for their effective role in physiological processes as well as their role in Reducing the soil pH and thus increasing the availability of microelements such as iron and zinc, which positively affects the vegetative characteristics, as nitrogen has an important role in the synthesis of organic compounds such as proteins, nucleic acids, amino acids, plant hormones and enzymes (Havan, 2005), and phosphorous has a role in the formation of a radical aggregate. Strong helps in the absorption of mineral substances that encourage growth (Hopkins and Huner, 2004), as well as the vital role of potassium in many physiological processes such as photosynthesis and respiration, as well as its role in metabolism and activation of many enzymes and encourage cell division and growth of plant tissues (Yassin, 2001) , in addition to its role in the osmotic regulation of the process of opening and closing stomata (Zhao et al., 2012) and thus increasing water absorption and Nutrients that activate the photosynthesis process and increase the elongation of cells and then increase the vegetative growth (Fawzy et al., 2007). As mentioned by Kandile et al. 2010)), the elements nitrogen and potassium help in the process of cell division and increase their numbers, which is reflected in the increase in vegetative growth. Also, spraying copper sulfate on lemon seedlings had an effect on a significant increase in the nutritional content of the vegetative group, due to the role played by the copper element in Improving growth indicators and giving plants a trait of tolerance and resistance to citrus gum disease, it was mentioned (Kuepper 2003) that foliar spraying with nutrients led to an increase in the content of these elements in the plant and also helped to increase the plant's resistance to disease by increasing the indicators of growth and plant health, as the element may have a role In activating the vital activities of the plant, including the process of absorbing water and nutrients, which helped reduce the percentage of wilting by increasing the soft weight of vegetative and root growths, and this was in line with (Hippler 2017), in addition to that copper provided the required plant's needs, which was positively reflected in the response Plants, as well as copper is essential for the work of some respiratory enzymes.

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