

## Effect of fertilization of Seaweed extracts and $\text{CuSO}_4$ on some vegetative growth indicators of *Citrus Limon L.* grafted seedlings on rootstock *aurantifolia*

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### Abstract

*Citrus aurantifolia* rootstock seedlings were treated with Seaweed extracts and copper sulfate. Seaweed extracts were added as a ground feed during the growing season at four levels (0, 10, 20, and 30 ml. seedling<sup>-1</sup>) and copper sulfate sprayed at four concentrations (15,10,5, 0 ml. L<sup>-1</sup>) alone or in combination with the control (watering with water only) during the growing seasons 2020-2021. The results showed that the treatment of seedlings grafted on rootstock *aurantifolia* with Seaweed extracts and spraying with copper sulfate affected (height, stem diameter, area Leaf, root length, and chlorophyll content of leaves (the results also showed that the addition of Seaweed extracts at a concentration of 30 ml overlapping with copper sulfate spraying at a concentration of 15 ml. L<sup>-1</sup> is recommended to improve the vegetative, root and nutritional growth of lemon citrus seedlings.

**Keywords:** *Citrus aurantifolia*, Seaweed extracts,  $\text{CuSO}_4$ . Copper sulfate.



## Introduction

Citrus belongs to the family Rutaceae, which grows in tropical and subtropical regions, 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 (10 and 16). Lemon (*Citrus limon* L.) Burm is one of the citrus species of the genus Citrus. The rootstock is located in the northeast of India and southwest of China (17), and most citrus species, including citrus, are propagated on different citrus rootstock, including aurantifolia. Several studies have indicated a mutual effect between rootstock and scion in citrus. The effect of rootstock in scion was more pronounced (3). Seaweed extracts are among 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 (14) 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 (23). 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 (10). 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 (2). 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(5). Electronic transport chain linking the two photochemical reaction systems of photosynthesis (21).

Based on the above and the lack of studies on it, the study aimed at the following:

- 1- Studying the effect of seaweed extracts and copper and the interaction between them on vegetative and root growth characteristics and nutritional status in order to obtain strong seedlings of local lemongrass grafted on two different rootstocks.
- 2- Study of the effect of rootstock used in grafting on the growth and nutritional status of growing lemongrass seedlings on rootstock aurantifolia.

## Materials and Methods

The research was carried out in a citrus production nursery in Al-Hindiya District - Holy Karbala Governorate, 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 extracts Algaton and copper sulfate  $\text{CuSO}_4$  (6.9% copper). In the growth of lemongrass seedlings grafted on rootstock and aurantifolia. 384 seedlings of homogeneous growth as much 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. A capacity of 10 kg of soil, and each seedling was planted in a plastic pot of 10 kg of soil. They were 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 irrigation and sometimes manual 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 *et al.* (22) and the results of the analysis are shown in Table (1).

**Table 1. Some chemical and physical properties of soil before planting.**

Feature	Unit	Values
Sand		82.0
Silt		7.0
Clay		1.0
<b>Soil components</b>		
soil texture	---	sandy silt
Ph	---	6.7
EC	$\text{ds m}^{-1}$	2.06
N	%	0.09
P	%	0.004
K	%	0.08
Ca	%	0.014
Mg	$\text{mmol L}^{-1}$	9
Fe	$\text{mg kg}^{-1}$	0.88
O.M	$\text{g kg}^{-1}$	3.09

#### Experience design

The treatments were distributed in the experiment using the Split-Split Plot Design system with three replications in the randomized complete block design (R.C.B.D). The main-plots represented four levels (0, 10, 20 and 30 ml. seedling<sup>-1</sup>) of ground fertilization with Seaweed

extracts It contains some major elements in addition to the organic matter (Table No. 2) adding a floor to each anvil as an injection into the soil of the anvil in the extension area of the seedling roots and with three additions and a period of 30 days between addition and another. The sub-plots have four levels (0,5,10,15 mg.

L<sup>-1</sup>) of foliar feeding with the copper element in the form of copper sulfate by spraying on the foliage of the leaves until complete wetness of the seedlings, with three additions and a period of 30 days between one addition and another. And the rootstock 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. (2). 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, 3 sprays were conducted on 15/8/2020 and 15/9/2020,

and the last spray was on 10/15/2020, as the ground plants were added with Seaweed extracts in the early morning and the plants were sprayed with copper sulfate three days later. 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. Components of Seaweed extracts (Algaton) 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-like biological 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:

Seedling height increase rate, main stem diameter increase rate, leaf area rate, root length increase rate, chlorophyll content of leaves (mg.100gm<sup>-1</sup> fresh weight).

## Results

### Plant height (cm)

It is noted from the results in Table (3) that there is a significant effect of the experimental factors and the interaction between them in this trait, and it was found that the seedlings that were added ground feeding at a concentration of 30 ml with Seaweed extracts significantly outperformed the plant height and scored the highest averages of 120.29 and 128.54 cm compared to plants resulting from seedlings The ones that were not fertilized and which recorded the lowest averages were 95.25 and 98.71 cm for the two

seasons of the experiment respectively, and a significant effect was shown in increasing the height of the plants by increasing the concentration of spraying with copper sulfate CuSO<sub>4</sub>, and the spraying treatment at a concentration of 15 ml. Which was sprayed with distilled water only and gave the lowest averages of 104.83 and 110.54 cm for the two seasons respectively. The ground with Seaweed extracts and rootstock had a significant effect in increasing the height of plants, and 30 ml treatment with rootstock aurantifolia produced the highest averages reached 134.25 and 140.33 cm, and the results showed a significant effect of the interaction between rootstock and copper sulfate, as it was significantly superior to rootstock aurantifolia x 15 mg.l<sup>-1</sup> CuSO<sub>4</sub> and produced the highest averages of 133.67 and 138.67 cm for the two seasons, respectively.

**Table 3. Effect of fertilizing with Seaweed extracts and copper sulfate and the interaction between them on plant height (cm).**

Algae Extract (ml.seedling <sup>1</sup> )	plant height (cm)								Average	
	First season				Second season					
	CuSO <sub>4</sub> (mgL <sup>-1</sup> )				CuSO <sub>4</sub> (mg.L <sup>-1</sup> )					
0	5	10	15	Average	0	5	10	15		
0	88.00	97.77	99.00	127.77	102.83	93.00	100.33	102.77	127.00	100.75
10	117.33	121.77	129.00	134.00	125.25	121.77	127.33	133.00	137.00	129.25
20	128.00	123.33	120.00	133.77	127.50	130.77	134.77	127.00	142.77	134.75
30	129.00	137.00	131.77	139.33	134.25	130.77	140.33	137.33	149.00	140.33
Average	110.33	119.77	121.17	133.77		121.50	125.42	124.50	138.77	
L.S.D. (P<0.05)	Algae Extract 0.84	CuSO <sub>4</sub> 2.92	Inter. 8.78			Algae Extract 2.56	CuSO <sub>4</sub> 1.25	Inter. 3.74		

The leaf area of the plant (cm<sup>2</sup>.plant<sup>-1</sup>)

We found through the results in Table (4) that the fertilizing treatment with Seaweed



extracts at a concentration of 30 ml had a significant effect on the leaf area of the plant, with the highest averages reaching 904.50 and 917.40 cm<sup>2</sup>. plant-1 for the two seasons, respectively, compared to the plants obtained from seedlings that did not fertilize, which recorded the lowest averages were 425.30 and 429.70 cm<sup>2</sup>. Plant-1 for both seasons of the experiment sequentially, and a significant effect was found in increasing the leaf area of plants, and the highest values were achieved after spraying with a concentration of 15 ml. L<sup>-1</sup> CuSo<sub>4</sub>, which amounted to 733.30 and 741.80 cm<sup>2</sup>. Plant<sup>-1</sup> for the two seasons of the experiment sequentially compared to the control treatment that was sprayed with

distilled water only, and it gave the lowest values of 625.60 and 632.90 cm<sup>2</sup>. Plant<sup>-1</sup> for the two seasons sequentially, the results of the same table indicate that the interaction between ground fertilization with Seaweed extracts and rootstock had a significant effect in increasing the leaf area and the treatment of 30 ml × rootstock aurantifolia produced the highest averages of 1012 and 1021 cm<sup>2</sup>. Plant<sup>-1</sup> for the two seasons of the experiment sequentially, and the results showed a significant effect of the interaction between rootstock and copper sulfate treatment, and the 15ml.L<sup>-1</sup> CuSo<sub>4</sub> × rootstock aurantifolia treatment produced the highest averages amounting to 837.50 and 843.10 cm<sup>2</sup>. plant<sup>-1</sup>.

**Table 4. Effect of fertilization with seaweed extracts and copper sulfate and the interaction between them on leaf area (cm<sup>2</sup>.plant<sup>-1</sup>) of rootstock aurantifolia.**

Algae Extract (ml.seedling <sup>-1</sup> )	leaf area (cm <sup>2</sup> .plant <sup>-1</sup> )									
	First season					Second season				
	CuSO <sub>4</sub> (mg.L <sup>-1</sup> )					CuSO <sub>4</sub> (mg.L <sup>-1</sup> )				
	0	5	10	15	Average	0	5	10	15	Average
0	394.3	408.0	406.3	408.3	404.3	396.0	412.3	412.7	403.0	408.0
10	607.0	697.3	747.0	791.0	723.1	760.0	707.0	703.0	790.7	730.2
20	822.0	893.0	977.3	981.7	918.0	828.3	908.0	989.0	988.0	928.3
30	994.7	1008.3	1029.0	1019.0	1012.8	1003.7	1023.0	1033.0	1020.7	1012.3
Average	720.7	771.3	703.7	733.3		732.9	769.7	711.8	741.8	
L.S.D. (P≤0.05)	Algae Extract 16.09	CuSO <sub>4</sub> 7.46	Inter. 21.81			Algae Extract 12.80	CuSO <sub>4</sub> 7.24	Inter. 20.46		

Diameter of the stem (mm)

Table (5) shows that there is a significant effect of the experimental factors and all their interactions on stem diameter, as it was found that plants treated with seaweed extracts at a concentration of 30 ml had a significant effect on the plant diameter and

gave the highest averages of 9.42 and 9.58 mm for the two seasons, respectively, compared to plants resulting from seedlings. that did not fertilize, which recorded the lowest averages of 6.16 and 6.48 mm for the two seasons of the experiment respectively, and it was also found that CuSo<sub>4</sub> spraying had a



significant effect on increasing the diameter of the stem. With distilled water only, it gave the lowest values of 7.51 and 7.76 mm for the two seasons respectively. Rootstock aurantifolia also showed a significant effect in stem diameter by giving the highest averages of 8.78 and 8.93 mm for the two seasons of the experiment respectively. The results of the

same table indicate that the interaction between ground fertilization with Seaweed extracts and rootstock It achieved a significant effect in increasing stem diameter, and the treatment of 30 ml × rootstock aurantifolia produced the highest averages of 10.64 and 10.73 mm for the two seasons of the experiment, respectively.

**Table 6. Effect of fertilization with Seaweed extracts and copper sulfate and the interaction between them on stem diameter (mm) for rootstock aurantifolia**

Algae Extract (ml.seedling <sup>1</sup> )	stem diameter (mm)									
	First season					Second season				
	0	CuSO <sub>4</sub> (mg.L <sup>-1</sup> )			Average	0	CuSO <sub>4</sub> (mg.L <sup>-1</sup> )			Average
	5	10	15		5	10	15			
0	7.51	7.76	7.96	7.74	7.51	7.76	7.96	7.74	7.74	7.96
10	7.76	7.96	8.20	8.11	7.76	7.96	8.20	8.11	8.11	8.20
20	8.11	8.20	8.78	8.69	8.11	8.20	8.78	8.69	8.69	8.78
30	8.20	8.78	10.64	10.64	8.20	8.78	10.64	10.64	10.64	10.64
Average	7.74	8.11	8.93	8.69	7.74	8.11	8.93	8.69	8.69	8.93
L.S.D. (P≤0.05)	Algae Extract 0.06	CuSO <sub>4</sub>			Inter. 0.44	Algae Extract 0.21	CuSO <sub>4</sub>			Inter. 0.13

Root length (cm)

Through the results in Table (6), it was found that there were significant differences for the single-experiment treatments and their interactions, as well as the triple interaction in this trait, as the plants treated with Seaweed extracts at a concentration of 30 ml excelled significantly in doubling the length of the roots and giving the highest averages of 14.29 and 16.78 cm for the two seasons, respectively. Compared with plants resulting from seedlings that did not fertilize, which recorded the lowest averages of 11.68 and 12.14 cm for the two seasons of the experiment respectively,

while CuSO<sub>4</sub> spraying had a significant effect on increasing the length of the roots. The comparison, which was sprayed with distilled water only, gave the lowest percentages of 12.24 and 14.07 cm for the two seasons sequentially. As for the rootstock, we found that rootstock aurantifolia was significantly superior in increasing the length of the roots. The highest percentages were 13.44 and 15.23 cm for the two seasons of the experiment sequentially. The results of the same table indicate that the interaction between Soil fertilization with Seaweed extracts and rootstock achieved a significant effect in increasing the number of roots, and a treatment of 30 ml × rootstock aurantifolia



produced higher average It reached 15.26 and 18.30 cm for both seasons of the experiment respectively, and the results showed a significant effect of the interaction treatment between rootstock

and copper sulfate, as it was significantly superior to rootstock aurantifolia with 15 mg.l<sup>-1</sup> CuSo<sub>4</sub> and produced the highest averages of 14.27 and 16.57 cm.



**Table 6. Effect of fertilization with seaweed extracts and copper sulfate and the interaction between them on root length (cm) of rootstock aurantifolia.**

Algae Extract (ml.seedling <sup>1</sup> )	root length (cm)									
	First season CuSO <sub>4</sub> (mgL <sup>-1</sup> )					Second season CuSO <sub>4</sub> (mg.L <sup>-1</sup> )				
	0	5	10	15	Average	0	5	10	15	Average
0	11.46	12.79	12.14	12.19	12.10	10.37	12.09	13.79	14.39	12.76
10	12.27	12.77	12.83	12.98	12.79	17.87	12.00	13.22	13.18	13.94
20	12.42	13.07	14.09	14.06	13.76	18.40	14.43	14.98	10.83	10.91
30	14.83	14.70	14.10	17.37	10.26	17.07	10.83	17.92	22.90	18.30
Average	12.70	13.43	13.30	14.27		10.00	13.84	14.90	17.07	
L.S.D. ( <i>P</i> ≤0.05)	Algae Extract 0.76	CuSO <sub>4</sub> 0.40	Inter. 1.007			Algae Extract 0.79	CuSO <sub>4</sub> 0.62	Inter. 1.68		

Chlorophyll content of leaves (mg.100gm<sup>-1</sup> fresh wt.)

It is noted from the results of Table (7) that the factors of the single experiment and their interactions, as well as the triple interaction, significantly affected the chlorophyll content of the vegetative complex. It was noted that the plants treated with fertilization with Seaweed extracts at a concentration of 30 ml had a significant effect in giving the highest averages, which amounted to 101.74 and 109.19 mg. 100 g<sup>-1</sup> for the two seasons, respectively, compared to plants produced from seedlings that were not fertilized, which recorded the lowest averages of 65.23 and 70.29 mg. 100 gm<sup>-1</sup> of the experimental seasons sequentially, while CuSO<sub>4</sub> spraying had a significant effect on increasing the plant's chlorophyll content, and the spraying treatment at a concentration of 15 ml.L<sup>-1</sup> showed the highest values of 87.85 and 93.52 mg. 100 gm<sup>-1</sup> for the two seasons of the experiment

sequentially compared to the control treatment that was sprayed with distilled water only, and it gave the lowest percentages of 79.25 and 84.97 mg. 100 gm<sup>-1</sup> for the two seasons respectively, and rootstock had a significant effect on chlorophyll content, it gave rootstock aurantifolia, which reached 77.75 and 82.77 mg. 100gm<sup>-1</sup> for two seasons, sequentially. The results of the same table indicate that the interaction between soil fertilization with Seaweed extracts and rootstock had a significant effect in increasing the chlorophyll content, and a 30 ml treatment with rootstock aurantifolia yielded 61.17 and 67.00 mg. 100 gm<sup>-1</sup> for both seasons of the experiment sequentially, and the results showed a significant effect of the interaction treatment between rootstock and copper sulfate, as it was significantly superior to rootstock aurantifolia with 15 mg.l-1 CuSo<sub>4</sub>, which were 73.61 and 79.21 mg. 100 gm<sup>-1</sup> for two seasons of the experiment sequentially.

**Table 7. Effect of fertilization with seaweed extracts and copper sulfate and their interaction on chlorophyll content (mg.100 gm<sup>-1</sup> fresh wt.) of rootstock aurantifolia.**

Algae Extract (ml.seedling <sup>1</sup> )	chlorophyll content (mg.100 gm <sup>-1</sup> fresh wt.)										
	First season					Second season					Average
	CuSO <sub>4</sub> (mgL <sup>-1</sup> )					CuSO <sub>4</sub> (mg.L <sup>-1</sup> )					
0	5	10	15	Average	0	5	10	15	Average		
0	67.80	70.77	72.73	73.07	71.17	73.80	75.77	77.73	79.90	77.00	
10	77.03	78.00	73.80	77.33	72.04	73.70	73.00	77.80	81.83	76.68	
20	79.40	81.70	83.47	87.70	82.79	83.73	84.70	89.27	92.93	87.76	
30	90.70	94.70	95.97	98.73	95.00	95.70	97.70	99.97	100.73	99.75	
Average	79.20	82.37	84.79	87.80		84.97	87.79	89.83	93.09		
L.S.D. (P≤0.05)	Algae Extract 0.30	CuSO <sub>4</sub> 0.19	Inter. 0.65			Algae Extract 0.29	CuSO <sub>4</sub> 0.27	Inter. 0.73			

## Discussion

The results mentioned in Tables 3-5 indicate that the seedlings grafted on aurantifolia rootstock were significantly superior to the grafted seedlings on the citrus rootstock in seedling height, number of leaves, leaf area of seedlings, main stem diameter of the scion, fresh and dry weight of shoots, and this may be attributed to the difference of rootstock in growth strength and physiological condition. And its effect on the strength of the growth of the developing grafts on it as a result of the difference in the absorption, transmission, accumulation and use of nutrients in the various growth processes that occur within the plant, which may be positively reflected in the physiological processes necessary for vegetative and root growth, and then its impact on the nature and strength of the growth of scion developing on it (9 and 21), that the difference between the studied rootstock in its effect

on the growth of grafts growing on it was consistent with what was found (1) when they studied three rootstocks of citrus fruits covered with lemon (8) in his study of Citrus aurantium rootstock and Vulkamariana scion on them by (20) when comparing five rootstocks of citrus scion with class orange. Salustiana that the rootstock differ with each other according to their genetic characteristics, as well as the vegetative and root growths are the most important features of the vital activity of the plant, and both are affected by the biological and physiological processes that occur in its different parts.

The results of vegetative and root growth indicators showed that the addition of Seaweed extracts caused significant differences in most of the vegetative characteristics compared with the comparison seedlings, which gave the lowest rates in the above-mentioned indicators, and the treated seedlings were at the level of 150 ml. Algazon-1 seedlings



of fertilizer are superior by giving them the highest rates, and the reason may be attributed to the increase in the vegetative growths under study with the use of increasing concentrations of Seaweed extracts, as its addition leads to providing the necessary nutrients in a ready-made way to the soil and the roots of seedlings can absorb and benefit from these nutrients, especially the major elements. For its effective role in physiological processes as well as its role in reducing soil pH and thus increasing the availability of microelements such as iron and zinc, which is positively reflected on 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 (13). Also, the seaweed extracts contain phosphorous, which plays a role in the formation of a strong root system that helps in absorbing minerals that encourage growth (12). An effective osmosis in the process of opening and closing stomata, and then increasing the absorption of water and nutrients and increasing the activity of the coffee process. Photosynthesis and its products, which are reflected in the increase in cell division and elongation and then increase in vegetative growth, as well as enter into 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 (25), as well as its role in regulation Osmosis for the process of opening and closing stomata (26) and thus increasing the absorption of water and nutrients that activate the photosynthesis process and increase the

elongation of cells and then increase vegetative growth (12). The results of this study agree with what was obtained by (4) when spraying the local lime seedlings with the nutrient solution Azuren Mix (7) using OMEX BIO-20 foliar fertilization on the local lime seedlings. In the same context, the increase of chlorophyll in the leaves of the seedlings that were included in the experiment and shown in the results of Table (5) may be attributed to the marine extract containing the necessary nutrients, especially nitrogen, phosphorous and potassium, which have a role in increasing the amount of chlorophyll, building proteins and increasing chlorophyll (13 and 18).

### Conflict of Interest

The authors have no conflict of interest.

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