

## Growth response of peppermint to nano copper and Algacifo fertilizer and their effect on the active substances

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### Abstract:

A study was carried out during the 2022 agricultural season in private nursery in Al-Qadisiyah Governorate, to study the response of peppermint plant growth to nano copper and (Algacifo) fertilizer and their effect on the active substances. The experiment was designed with randomized complete blocks, with three replications. The experiment included 4 concentrations of nano copper (0, 1, 2, and 3) g L<sup>-1</sup> and 4 concentrations of Algacifo fertilizer (0, 2, 4, and 6) ml L<sup>-1</sup>. The results showed that the concentration of 1 g L<sup>-1</sup> of nano copper in the most of the studied traits was superior in a significant increase (number of branches and leaves, total leaves area, and percentage of Menthone), while the highest plant height was at 2 gL<sup>-1</sup> concentration, and the concentration 3 g L<sup>-1</sup> achieved the highest percentage of Menthol. The impact of nano copper in all its concentrations was negative in the percentage of Limonene. The concentration of 4 ml L<sup>-1</sup> of Algacifo was superior in recording the number of branches, and the concentration of 6 ml L<sup>-1</sup> was distinguished in achieving the highest plant height, the number of leaves, the largest total leaves area, and the highest percentage of Menthol, and Algacifo fertilizer had no significant impact on the percentage of Menthone, While its impact was negative in the percentage of Limonene.

The two interactions of the two factors of the study were superior to the mix of 2 g L<sup>-1</sup> of nano copper with 6ml L<sup>-1</sup> of Algacifo fertilizer in achieving the highest plant height. In contrast, the mix of 1g L<sup>-1</sup> of nano copper with 4 ml L<sup>-1</sup> of the Algacifo had the highest number of branches and the highest percentage of Menthone. The mix of 1g L<sup>-1</sup> of nano copper with 6 ml L<sup>-1</sup> of Algacifo gave the most significant number of leaves and the most extensive total leaves area. The mix was characterized by 3g.L<sup>-1</sup> of nano copper with 6 ml L<sup>-1</sup> of Algacifo to achieve the highest percentage of Menthol. All the combinations used caused a significant decrease in the percentage of Limonene.

**Keywords:** Nano Copper, Algacifo. peppermint, Menthol, GCMS.

### Introduction:

Medicinal plants are a huge product pharmaceutical drug [1] and the genus *Mentha* has commercial value due to its aromatic, antioxidant, antidiabetic, and anti-inflammatory properties. In general, the presence of biologically active chemicals is the main reason for this pharmacological activities, including 25 species, including Peppermint. *Mentha piperita* L [2,3]. Peppermint belongs to the genus *Mentha*, the family Lamiaceae; it is considered one of the impactful medicinal plants, it is a hybrid of

spearmint and water mint, it is commonly used as an essential oil, it is tried to get rid of abdominal pain associated with irritable bowel syndrome and has a restful impact on the smooth muscles of the organ, digestive and helpful for gastric hypermotility, functional dyspepsia. This oil contains menthol, menthone, menthyl esters, acetaldehyde, and other active compounds [4].

The agricultural sector faces a significant problem related to production with the increase in the population and the deterioration of agricultural lands [5]. The

adoption of more efficient tools and technology can lead to sustainable agriculture, nano-fertilizers can control the release of nutrients and provide the right amount of nutrients to crops in the right proportion, enhancing yield while maintaining environmental safety[6]. Nano-fertilizers, due to its high surface area to volume ratio are more impactful than most of the latest polymeric-type conventional fertilizers, They could also allow the slow release and efficient nutrient uptake by crops, this technology, therefore, offers excellent platform for sustainable and novel nutrient delivery systems that penetrate the nano porous surfaces of plants encapsulated nanoparticles, nanoclays and zeolites, increased efficiency in terms of fertilizer application, restoration of soil fertility and plant health and reduction of environmental pollution and agroecology degradation [7].

Copper is the micronutrients needed for plant it plays a role in photosynthesis and respiration through the transfer of electrons to oxygen and helps form lignin in the cell walls, which supports the plant, high doses impactively inhibit plants' growth and show anti-bacterial and anti-fungal activities [8]. And 50% of agricultural soils contain small amounts of copper available to plants, as copper moves from roots to shoots through xylem and cannot be easily re-transferred by the bark, symptoms of deficiency appear in young leaves, which weakens crop yield and nutritional quality, both copper uptake and efficient utilization are essential to plant quality [9].

Excessive use of chemical fertilizers causes severe damage to the physical and chemical properties of soil, the findings of [10] indicate the benefits of organic matter in organic fertilizers, which can enhance their use and non-use of chemical fertilizers, as the organic matter in organic fertilizers has flexibility in The ability to replace nitrogen, phosphorous and potassium in chemical fertilizers, and therefore the provision of

organic fertilizer that contains a sufficient amount of organic matter and bacteria reduces the use of chemical fertilizers as well as the cost of production [11]. [12] show that seaweed extract used in organic fertilizers is more impactful in increasing vegetative growth and plant production.

Because of the mint plant's medical importance, its multiple and broad uses, and the role of its active substances, especially volatile oils, the study aims to respond to spraying with nano copper and Algacifo organic fertilizer and their impact on the active substances.

### Materials and Methods:

The study was executed in pots with a capacity of 20 kg in the agricultural season 2022 in one of the private nurseries in Al-Qadisiyah Governorate, with coordinates (longitude 44.9060 and latitude 32.0112). the pots soil was analyzed, and Table (1) shows its properties physical and chemical. The experiment was designed in Randomized Completely Blocks with a factorial organization; it included four concentrations of chelated nano copper (0, 1, 2, and 3) g L<sup>-1</sup> and four concentrations of fertilizer Algacifo (0, 2, 4, and 6) ml L<sup>-1</sup> and in Three replicates. The seedlings were planted on 20/2/2022, as three plants were grown in one pot, each seedling was 15 cm high, and the fertilizer was added in two doses, the first of Nano Chelated Copper imported from Al-Khadra Company for Nano Fertilizer in Iran 15 days after the date of planting on 5/3/2022 (a powder containing 9% chelated copper, soluble in water and absorbable at pH (3-11), weigh each concentration separately and place it in a 1-liter hand sprayer and complete the volume by adding distilled water. On the next day, on 6/3/2022, the organic fertilizer Algacifo imported from Bluefield Offshore Lebanon Company was added (it is a liquid fertilizer extracted from brown seaweed, consisting of 2% organic nitrogen, 10% organic carbon, 50% the molecular mass of the organic matter)

by measuring each concentration individually and placing it in a 1-liter hand sprayer and completing the volume by adding distilled water.

The second dose application was a month after the first foliar application. The agricultural processes had been done, including irrigation and weeding manually, according to the needs of the pots.

The studied traits were measured two months after planting the seedlings on 20/4/2022 by taking the average of 5 random plants from each experimental unit for each of the following traits: Plant height (cm) using the measuring tape from the soil surface to the top of the plant, and the number of branches (branch plant<sup>-1</sup>) as the number of branches from each treatment for each replicate was calculated, and then the average number of branches for each plant was extracted. And leaf area (cm<sup>2</sup> plant<sup>-1</sup>) by multiplying the number of leaves by the area of one leaf per plant was measured using Digimizer software in Windows 7 operating system [13]. The oil was extracted from the leaves according to the method of [14], as 40 g of dried and ground leaves were taken, then placed in a 1000 ml water beaker with 600 ml of distilled water and subjected to water distillation for 3 hours, then collected the essential oils in small bottles and dried them over sulfate Anhydrous

sodium, one drop of oil was dissolved in 2 ml of ethyl acetate and cooled until analysis. Then one microliter of it was injected into the GCMS-QP2010 Ultra Shimadzu device, which includes an automatic identification unit based on mass spectra under the following conditions: Separation column consisting of 100% dimethyl polysiloxane with dimensions (1 µm x 0.25 mm x 30 m), helium gas carrier with a flow rate of 1 ml min<sup>-1</sup> and the temperatures of the injector and ion source are 250°C and 220°C, respectively. The oven temperature was programmed automatically to obtain a temperature gradient, starting from 50° C (an equal temperature for 3 minutes) and increasing 8° C every minute up to 250° C. The components were determined using the database of the National Institute of Measurement and Technology by comparing the resulting spectrum of the component. Anonymous with known stored components in the National Institute of Standards and Technology (NIST) Library. Depending on the importance of the active compounds and the highest rate, the following compounds (Menthol, Menthone and Limonene) were selected, and the rate of these materials represented their percentage of the substances in the extract (not their concentration or percentage in the plant) depending on the type of column used in the GCMS device and the type of extract.

**Table (1): Part of properties physical and chemical of potted soil.**

Some soil properties	The value	Unit
Soil reaction pH	---	8.14
Electrical Conductivity Ec (1:1)	ds m <sup>-1</sup>	2.1
Soil organic matter (SOM)	g kg <sup>-1</sup> soil	3.42
Ready Nitrogen	mg kg <sup>-1</sup> soil	20.14
Ready Phosphorous		6.87
Ready Potassium		187.51

Sand	g kg <sup>-1</sup> soil	225
Silt		350
Clay		425
soil texture	Clay Silt Loam	

## Results:

### 1- plant height (cm)

Table (2) shows the significant impact of the study factors and their interactions on plant height, as the impact of using concentrations (1 and 2) g L<sup>-1</sup> of nano-copper significantly increased plant height, which reached (45.17 cm and 53.17 cm), respectively. In contrast, the concentration of 3 g L<sup>-1</sup> caused a significant decrease, reaching 37.58 cm, compared to the control treatment of 40.58 cm.

Algacifo fertilizer levels caused a significant gradual increase that reached a maximum at 6 ml L<sup>-1</sup> concentration came 51.58 cm compared to the control treatment of 35.58 cm.

Two interaction factors studied were significant, as nano copper caused a substantial increase at each concentration of Algacifo fertilizer for most combinations; the maximum was 61.67 cm when the combination of 2 g L<sup>-1</sup> for all combinations the comparison treatment of 33.33 cm.

**Table (2) Impact of nano copper and Algacifo and their interactions on height (cm) of *Mentha piperita* L.**

nano copper concentrations g L <sup>-1</sup>	Algacifo fertilizer concentrations ml L <sup>-1</sup>				Average impact of nano copper
	0	2	4	6	
0	33.33	36.67	43.33	49.00	40.58
1	36.00	42.33	48.33	54.00	45.17
2	42.67	51.67	56.67	61.67	53.17
3	30.33	36.33	42.00	41.67	37.58
Average impact of Algacifo fertilizer	35.58	41.75	47.58	51.58	
L.S.D 0.05	1.62				1.62
two interactions between nano copper and Algacifo fertilizer	3.23				

## 2- Branches number (branch plant<sup>-1</sup>)

Table (3) indicates the significant impact of nano copper, as different concentrations varied. Hence, the concentration of 1 g L<sup>-1</sup> caused a considerable increase and gave the

most branches number, reaching 10.17 branches plant<sup>-1</sup>, while concentration of 3 g L<sup>-1</sup> led to a significant decrease of 6.50 cm. The concentration of 2g L<sup>-1</sup> did not differ from the control treatment, which was 8.50 branch plant<sup>-1</sup>.

**Table (3) Impact of nano copper and Algacifo and their interactions on branches number (Branch plant<sup>-1</sup>) of *Mentha piperita* L.**

nano copper concentrations gl <sup>-1</sup>	Algacifo fertilizer concentrations mll <sup>-1</sup>				Average impact of nano copper
	0	2	4	6	
0	4.33	7.67	10.67	11.33	8.50
1	5.33	10.00	12.00	13.33	10.17
2	6.67	7.67	9.67	11.33	8.83
3	5.00	6.33	7.67	7.00	6.50
Average impact of Algacifo fertilizer	5.33	7.92	10.00	10.75	
L.S.D 0.05	0.84				0.84
two interactions between nano copper and Algacifo fertilizer	1.68				

The use of organic fertilizer was significant in the number of branches of the plant, so it reached the highest number at the concentration of 6 ml L<sup>-1</sup>, which did not differ significantly from the concentration of 4 ml L<sup>-1</sup>, given (10.75 and 10.00) branch plant<sup>-1</sup>, respectively, compared to the treatment comparison amounted to 5.33 branch plant<sup>-1</sup>.

Two interactions of the two factors of the study was significant in rising branches number for all combinations, reached a maximum in the two combinations: 1 g L<sup>-1</sup> of nano copper with (4 and 6) ml L<sup>-1</sup>, which did not differ significantly from each other, as it gave (12.00 and 13.33) branch plant<sup>-1</sup>, respectively, compared to all combinations and the control treatment, gave 4.33 branch plant<sup>-1</sup>.

## 3- Leaves number (leaf plant<sup>-1</sup>)

Table (4) shows a significant impact of nano copper in increasing the number of leaves is apparent, as the most number was 66.33 leaf plant<sup>-1</sup> at 1 g L<sup>-1</sup> concentration, compared to 51.25 leaf plant<sup>-1</sup> of control treatment. The increase in leaves number was gradual with the rise in the concentrations of the Algacifo fertilizer and reached their maximum at concentrations 4 and 6 ml L<sup>-1</sup>, that did not differ significantly from each other, as they gave (61.67 and 65.17) leaf plant<sup>-1</sup>, respectively, compared to 49.92 leaves plant<sup>-1</sup> of comparison treatment.

Interaction was significant in the increase leaves number for most of the combinations, that reached a maximum of

81.67 leaves plant<sup>-1</sup> when the mix of 1g L<sup>-1</sup> nano copper with 6ml L<sup>-1</sup> of Algacifo fertilizer compared to all combinations and with 44.00 leaf plant<sup>-1</sup> of comparison treatment.

**Table (4) Impact of nano copper and Algacifo and their interactions on leaves number (leaf plant<sup>-1</sup>) of *Mentha piperita* L.**

nano copper concentrations g L <sup>-1</sup>	Algacifo fertilizer concentrations ml L <sup>-1</sup>				Average impact of nano copper
	0	2	4	6	
0	44.00	50.00	52.33	58.67	51.25
1	51.67	59.67	72.33	81.67	66.33
2	54.00	61.67	65.67	67.67	62.25
3	50.00	50.33	56.33	52.67	52.33
Average impact of Algacifo fertilizer	49.92	55.42	61.67	65.17	
L.S.D 0.05	3.77				3.77
two interactions between nano copper and Algacifo fertilizer	7.54				

#### 4- Total leaf area (cm<sup>2</sup> plant<sup>-1</sup>)

**Table (5) Impact of nano copper and Algacifo and their interactions on the total leaf area (cm<sup>2</sup> plant<sup>-1</sup>) of *Mentha piperita* L.**

nano copper concentrations g L <sup>-1</sup>	Algacifo fertilizer concentrations ml L <sup>-1</sup>				Average impact of nano copper
	0	2	4	6	
0	207.5	263.5	322.2	369.4	290.6
1	275.4	335.7	411.9	493.9	379.2
2	299.4	394.3	407.1	429.5	382.6
3	210.7	216.3	233.1	212.6	218.2
Average impact of Algacifo fertilizer	248.3	302.4	343.6	376.4	
L.S.D 0.05	24.78				24.78
two interactions between nano copper and Algacifo fertilizer	49.55				

Table (5) indicates the significant impact of nano copper on the total leaf area of the plant, which reached a maximum area of  $382.6 \text{ cm}^2 \text{ plant}^{-1}$  at a concentration of  $2 \text{ g L}^{-1}$ , which did not differ significantly from  $379.2 \text{ cm}^2 \text{ plant}^{-1}$  resulting from the use of  $1 \text{ g L}^{-1}$  concentration, compared to  $290.6 \text{ cm}^2 \text{ plant}^{-1}$  of control treatment. The benefit of Algacifo fertilizer was a gradual and significant increase the total leaf area for each of its concentrations which reached a maximum of  $376.4 \text{ cm}^2 \text{ plant}^{-1}$  at  $6 \text{ ml L}^{-1}$  concentration compared to  $248.3 \text{ cm}^2 \text{ plant}^{-1}$  of control treatment.

The binary interaction of the two factors of the study was significant, as the largest total leaf area was  $493.9 \text{ cm}^2 \text{ plant}^{-1}$  when the mix of  $1 \text{ g L}^{-1}$  of nano copper with  $6 \text{ ml L}^{-1}$  of Algacifo fertilizer, that differed significantly from all other combinations and from the comparison treatment that was given  $207.5 \text{ cm}^2 \text{ plant}^{-1}$ .

## 5- Percentage (%) of Menthol

Table (6) shows the significant impact of nano copper in the percentage of Menthol, as the concentrations (2 and 3)  $\text{g L}^{-1}$  caused a substantial increase of it, that reached a maximum of 42.40% at the concentration 3  $\text{g L}^{-1}$ . In contrast, did not differ the concentration 1  $\text{g L}^{-1}$  from the 38.56% of comparison treatment. The significant impact of Algacifo fertilizer reaching the highest percentage 41.61% at the concentration of 6  $\text{ml L}^{-1}$ , that differed significantly from the 38.64% of control treatment and the rest concentrations.

The binary interaction between nano copper and the Algacifo fertilizer showed a significant increase that varied in different combinations, reaching a maximum of 44.86% when the combination of 3  $\text{g L}^{-1}$  of nano copper with 6  $\text{ml L}^{-1}$  of Algacifo fertilizer compared to 36.52% of control treatment.

**Table (6) Impact of nano copper and Algacifo and their interactions on percentage (%) of Menthol of *Mentha piperita* L.**

nano copper concentrations $\text{g L}^{-1}$	Algacifo fertilizer concentrations $\text{ml L}^{-1}$				Average impact of nano copper
	0	2	4	6	
0	36.52	38.00	40.00	39.72	38.56
1	38.75	38.81	40.29	39.67	39.38
2	38.16	40.10	38.94	42.17	39.84
3	41.13	42.30	41.30	44.86	42.40
Average impact of Algacifo fertilizer	38.64	39.80	40.13	41.61	
L.S.D 0.05	0.96				0.96
two interactions between nano copper and Algacifo fertilizer	1.93				

## 6- Percentage (%) of Menthone

Table (7) indicates the substantial impact of nano copper on the percentage of Menthone, as the concentration was caused by 1 g L<sup>-1</sup>, which was a substantial increase in the rate amounted to 33.21% compared to 28.14% of control treatment. In contrast, the two concentrations (2 and 3) g L<sup>-1</sup> did not differ from each other and the comparison treatment. Algacifo fertilizer was not significant in its impact on this trait.

The two interaction between nano copper Algacifo had a significant impact in rising the percentage of most of the combinations, as the highest percentage of Menthone reached 34.99% when the mix of 1 g L<sup>-1</sup> of nano copper with 6 ml L<sup>-1</sup> of Algacifo, which did not differ significantly from the two combinations of 1 g L<sup>-1</sup> of nano copper with (2 and 4) ml L<sup>-1</sup> of organic fertilizer gave 32.95% and 33.69%, respectively, and compared to 26.95% of control treatment and with the rest combinations.

**Table (7) Impact of nano copper and Algacifo and their interactions on percentage (%) of Menthone of *Mentha piperita* L.**

nano copper concentrations g L <sup>-1</sup>	Algacifo concentrations ml L <sup>-1</sup> fertilizer				Average impact of nano copper
	0	2	4	6	
0	26.95	28.65	27.34	29.63	28.14
1	31.18	32.95	33.69	34.99	33.21
2	29.89	29.14	29.75	26.86	28.91
3	28.19	28.65	30.57	25.27	28.17
Average impact of Algacifo fertilizer	29.05	29.85	30.34	29.19	
L.S.D 0.05	N.S				1.37
two interactions between nano copper and Algacifo fertilizer	2.75				

## 7- Percentage (%) of Limonene

Table (8) indicates the negative impact of all concentrations of nano copper on the percentage of Limonene. The significant decrease was graded with increasing concentrations, reaching a maximum of 1.708% at 3 g L<sup>-1</sup> concentration compared to 4.435% of control treatment. Algacifo

fertilizer also had a negative impact on the percentage of Limonene, as it recorded the lowest percentage of 2.473% at 6 ml L<sup>-1</sup> concentration, which did not differ significantly from the 2.648% obtained from using the concentration of 4 ml L<sup>-1</sup> of Algacifo fertilizer and compared to the control treatment that It gave 3.778%.



**Table (8) Impact of nano copper and Algacifo and their interactions on Percentage (%) of Limonene of *Mentha piperita* L.**

nano copper concentrations g L <sup>-1</sup>	Algacifo fertilizer concentrations ml L <sup>-1</sup>				Average impact of nano copper
	0	2	4	6	
0	5.490	4.120	4.200	3.930	4.435
1	4.433	3.850	2.150	1.740	3.043
2	3.450	2.780	2.640	2.450	2.830
3	1.740	1.720	1.600	1.770	1.708
Average impact of Algacifo fertilizer	3.778	3.118	2.648	2.473	
L.S.D 0.05	0.488				0.488
two interactions between nano copper and Algacifo fertilizer	0.976				

The two interactions were negative in the percentage of Limonene, where the lowest percentage was 1.648%, when the combination of 3 g L<sup>-1</sup> of nano copper with 4ml L<sup>-1</sup> for organic fertilizer Algacifo, which did not differ significantly from (1.740%, 1.720%, 1.770%, 2.150, 1.740% and 2.450%) obtained from the use of combinations (3g L<sup>-1</sup> of nano copper with (0, 2, and 6) ml L<sup>-1</sup> of organic fertilizer) and combinations (1g L<sup>-1</sup> of nano copper with (4 and 6) ml L<sup>-1</sup> of organic fertilizer) and the combination of 2g L<sup>-1</sup> of nano copper with 6ml L<sup>-1</sup> of Algacifo fertilizer, respectively.

### Discussion:

The results showed that the concentrations of nano copper and the Algacifo fertilizer and their interactions significantly affected vegetative growth characteristics and the production of active

substances. The rise in most of the studied traits (branches number, leaves number, total leaf area and percentage of Menthone) using nano copper at a concentration of 1g L<sup>-1</sup> (Tables- 3, 4, 5 and 7) is because treatment with nano copper leads to an increase in The photosynthetic pigments (chlorophyll, carotene and anthocyanins), which in turn help in plant growth [15], which led to an increase in the photosynthesis process and thus an increase in the vegetative total, including branches number, leaves number, the total leaf area of the plant and the proportion of the compound Menthone, as well as Increasing plant height and percentage of Menthol using concentrations (2 and 3) g L<sup>-1</sup>, respectively (Table -2 and 6). These results are in agreement with [16] in their study on the mustard plant *Brassica juncea*, who indicated that the response of the plant to copper nanoparticles used as a spray on the leaves depends on the concentration and causes an

increase in stomatal conductivity, which increases the rate of gas exchange, which improves photosynthesis and leads to the production of biomass. Higher and more productive, these molecules interact with meristems that lead to conductive biochemical pathways to enhance growth traits. While it had a negative impact on the percentage of Limonene (Table 8), this may be due to the increase in the production of Menthol and Menthone compounds at the expense of Limonene (Tables - 6 and 7).

The rise in plant height, branches number, leaves number, total leaf area, and percentage of Menthol using Algacifo fertilizer (Tables- 2, 3, 4, 5 and 6) is due to the role of organic nitrogen, which contributed to the production of the plant hormones IAA and GA3 in the mint plant, which It leads to excitation cell division and elongation and rising leaves and branches number and the rest of the studied growth indicators, as well as the role of carbon and organic matter, which causes an increase in the photosynthesis process and thus plant growth, and this is consistent with [17] in his study on the mint plant *Mentha piperita*, as he indicated The use of organic fertilizer led to the improvement of plant growth, the formation of endogenous plant hormones and the formation of the essential oil. The organic fertilizer Algacifo had no significant impact on the percentage of Menthone (Table-7). And it had a negative impact on the percentage of Limonene compound (Table- 8).

Most of the combinations used between study factors positively increased studied growth indicators, as nano copper, organic nitrogen, and organic carbon had a complementary role in plant growth. The emergence of combinations with the same moral impact indicates the possibility of using alternative blends according to the available ones and the economic situation, while the impact of all combinations was negative on the percentage of Limonene, and the reason for this may be due to the formation of other

compounds in the plant at the expense of this percentage.

### Conclusions:

The results of our current study showed the positive role of copper nanoparticles and Algacifo fertilizer in the growth of the mint plant, which varied according to the different concentrations of the study factors, so the nanoparticles behaved similarly to plant hormones, as low concentrations had the most significant role in promoting plant growth. At the same time, high concentrations of it caused an increase in some active plant compounds, which opens the way for broader studies of these molecules and their role in the development of different types of plants. In contrast, the Algacifo fertilizer stimulated plant growth in most of the concentrations used. The combinations between the study factors positively affected on the studied growth indicators.

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