Caspian Journal of Environmental Sciences

Online ISSN: 1735-3866
Print ISSN: 1735-3033

Effect of fulvic acid and chitosan in the growth of Roselle plants, *Hibiscus sabdarrifa* L and their nutrient content

Hala Jumaah Asree [1*], Noor Emad Kareem[1], Ahmed Ali Khirallah[2]

1. Faculty of Biotechnology, Al-Qassim Green University, Babylon, Iraq
2. College of Medicine, University of AL- Ameed, Karbala, PO Box189, Iraq

** Corresponding author's E-mail: hala_halaali@yahoo.com*

ABSTRACT
A factorials experiment was carried out in one of the fields of Al-Mashrooa City (45 km north of Babylon Province) for the agricultural season of 2018, with the aim of evaluating the effect of the treatment with several levels of organic acid (fulvic) and the chitosan on the growth of the *Hibiscus sabdariffa* L. and the nutrient content of its leaves. The first factor is the use of four levels of organic acid- fulvic - (1, 2, 3, and 4 mL L^{-1}). In addition to the comparison treatment, the second agent sprayed the chitosan with three concentrations (1, 3 and 5 mL L^{-1}), followed by the design of Random Complete Blocks Design (RCBD), in the research experiment and three replicates per treatment. The two solutions sprayed led to a significant increase in all vegetative growth indices and the leaf nutrient content. The highest concentration of the leaves was higher in the following studied traits (leaf area 50.34 cm^2, dry weight of vegetative total 572.4 g, leaf content of chlorophyll 52.13 SPAD, leaf content of iron 69.88. The interaction of 3 mg L^{-1} fulvic acid with the highest concentration of chitosan was superior in the following studied traits: plant height 112.33 cm, number of vegetative branches 32.67 branches, leaf content of nitrogen and potassium 1,487%, 1.920%, while the overlap of 2 mg L^{-1} Fulvic acid with the highest concentration of chitosan in the phosphorus content of leaves was 0.287%. The calcium content of leaf exceeded the overlap of 3 mg L^{-1} fulvic acid with 3 mg L^{-1} chitosan at a rate of 3.913%. The lowest results were recorded when comparison of the treatments and all the characteristics studied in the experiment.

Keywords: *Hibiscus sabdariffa* L., Fulvic acid, Chitosan, Leaf nutrient contents.
Article type: Research Article.

INTRODUCTION
Hibiscus sabdariffa L. belongs to the Malvaceae family of subtropical plants. Equatorial Africa is considered to be its home country and is cultivated in many tropical countries. In Iraq, its cultivation is spread in the southern regions, especially in Qadisiyah (Imran 1988). It is classified as a perennial plant (1- 2 years) and lives in different soils and has the ability to tolerate high temperatures. In total, the plant life cycle takes place from seed germination to fruiting for about 4-6 months (Galaudu 2006), which is grown for fruit leaves. The preparation of refreshing drinks with a sour taste is an important source of medical-induced calcosides in decreasing high blood pressure, strengthening the heart muscle, calming nerves and reducing blood viscosity (Bale 2009; Rao 1996). In the food industry, its leaves are used in the preparation of jams, sweets and food preservation because they contain a high percentage of phenolic compounds, organic acids and some important vitamins such as A, C in addition to iron, calcium, anthocyanin's, niacin and riboflavin (Azooz 2009). Recent research has been directed towards clean agriculture to reduce the harmful effects of chemical pesticides and their harmful effects on health.

Caspian Journal of Environmental Sciences, Vol. 20 No. 2 pp. 373-383 Received: June 20, 2021 Revised: Aug. 09, 2021 Accepted: Nov. 10, 2021
DOI: 10.22124/CJES.2022.5579

Publisher: University of Guilan, Iran

The organic renaissance was one of the most important strategic steps taken in this field because of its role in improving production and increasing its significant impact on improving the physical and chemical properties of the soil, biomass increase, and increase the effectiveness of vital activities of microorganisms (Ali 2012). Several studies have pointed to the importance of humus organic acids in improving the quantity and quality of Roselle, including fulvic acid. It is characterized by its low molecular weight, so it is easy to absorb by the plant even in the conditions of acid soils and it stimulates and activates plant hormones. It increases plant growth and harvest. It is an environmentally safe, odorless, non-toxic compound that reduces pollution of groundwater and the spread of fungal and bacterial diseases, since it is free from heavy elements (Naimi 1999). Thus, Majul *et al.* (2012) reported that the treatment of Roselle plants with different concentrations of humic acid caused a significant response to vegetative growth and quantity. Al-Tahafi *et al*. (2015) showed that the treatment of organic matter by spraying on the vegetative total of Roselle plants contributed to a significant increase in growth indices and yield in high salinity soils. In the case of chitosan, it is a bio-functional compound characterized by its non-toxicity and has no local or general effects in living tissues. There are some reports about using chitosan on some agricultural products (Ghasemnezhad *et al.* 2010; Rostamzad *et al.* 2019; Zahmatkesh *et al.* 2020). It has the ability to inhibit the growth of fungi, since it stimulates the chitinase, a defence enzyme which elevates the resistance of the plant to withstand environmental pollutions and face different stress conditions, being strengthens its growth (Devlieghere *et al.* 2004). The study aims to investigate the effect of the combined interaction between humic fulvic acid and chitosan in increasing the growth indices of Roselle plants and the content of their leaves of nutrients.

MATERIALS AND METHODS

The research experiment was carried out in the Musayyib project area (45 km north of Babylon Governorate) to investigate the effect of the treatment with different levels of organic matter represented by humic fulvic acid with spraying in several concentrations of chitosan in the growth of Roselle plants and their nutrient contents. The experimental soil was plowed by two orthogonal plows, then it was smoothed and levelled, and divided into three sectors (each sector included 20 experimental units, each area of 9 m^2). As the experimental unit included 3 lines and the distance between one and another line followed by 75 cm (Hassoun 2017). The seeds of the variety Sabdariffa, which is commonly grown in Iraq, obtained from a farmer in Diwaniyah city on 12/4/2021. The seeds were planted in pits at the top third of the line. The distance between one pit to the other was 50 cm and alternately on both sides of the meadow by 3 seeds in one pit at a depth of about 5 cm. The plants were irrigated first without immersion until germination was completed. Once the seedlings reached a height of 10-15 cm, thinning and grafting were carried out. Then the plants were irrigated as needed, with all crop service operations such as hoeing, weeding and combating whenever needed. Fulvic acid fortified with calcium was added and its properties are shown in Table 1. In 10/5/2021, it was added directly to the field soil 4 times the difference between addition and another followed by one month. In the case of chitosan compound, it was sprayed 3 days after the terrestrial addition of fulvic acid with a hand sprinkler in the morning to avoid high temperatures and the dryness of the solution on the plant. It was prepared by adding 1, 3 and 5 g of chitosan according to the concentrations used in the experiment with adding 0.5 mL acetic acid then completing the volume to 100 mL by adding distilled water. It was carried out by adding a few drops of clear liquid to the solution as a spreader for the purpose of increasing the surface tension of water during spraying (Abbas, 2016). In addition, guard lines were placed between the experimental units of the treatments to prevent overlapping of the spray treatments among them.

Table 1. Ingredients of organic solution (Tarasoil Calcio).

Element	Total nitrogen (%)	Nitric acid (%)	CaO (%)	Fulvic acid (%)
percentage	8.5	8.5	16.0	24.0

Treatments and experimental design

A factorials experiment (5 × 4) was conducted according to the design of the complete random sectors (RCBD = Random Complete Blocks Design) and three replicates per treatment. The first factor represented the addition of organic acid (fulvic acid) at four levels in addition to the comparison of treatments and its symbol (V_0, V_1, V_2, V_3 and V_4), while the second factor was sprayed with the chitosan compound three times (1, 2, 3 and 4) and concentrations of 1, 3, and 5 mL L^{-1} in addition to the comparison of treatments and their symbols (C0, C1, C2 and C4). The averages were compared using the least significant difference test (LSD). Least Standard Different is under a probability level of 5% and the results were analysed according to the statistical program GenStat 2008 (Sahoki & Waheeb 1990).

Examined characteristics

First / vegetative growth characteristics: Examined at the flowering stage after taking 5 plants from each experimental unit to study the following **characteristics**:

1 - Plant height (cm): This character was measured by a metric measure starting from the surface of the soil to the top of the plant.

2- Number of branches of vegetation

3- Leaf area (cm^2): Measured by a (digital planimeter) device and repeated the process three times after the average and extracted the area of leaf per treatment.

4. Leaf chlorophyll content (SPAD): The chlorophyll content of leaves was estimated by Chlorophyll meter Model SPAD - 502 after the third spraying of each of the organic acid and the chitosan compound for five fully-expanded leaves of the plant and then according to its average (Williams & Jemison 2006).

5 - Dry weight of the shoot system (g): Five plants were randomly taken from each experimental unit and dried in the direct sunlight for 10 days until the total drought and then the rate of weight by a sensitive balance.

Second / Chemical characteristics: The percentage of nutrients in the leaves in each experimental unit was estimated after washing them well to get rid of plankton and dirt. Then they were placed in perforated bags and dried in an electric oven at 70 °C until the weight was stable. Then, they were ground using an electric grinder and 0.2 g of dry leaf powder was taken and digested by adding 3 mL Sulfuric acid and 1 mL biochloric acid in the digestive system at a temperature of 240 °C for two hours. After obtaining the filtrate, the volume was reached to 50 mL and placed in plastic containers to determine the following elements (Gresser & Parsons 1979):

1. Nitrogen (%): Determined using the Microkjedhal method according to the methodology of Al-Sahhaf (1989).

2. Phosphorus (%): It was determined by the soft digestion method using ammonium molybdate and ascorbic acid by colorimetric method using a spectrophotometer (John 1970).

3. Potassium (%): Estimated using a flame-photometer according to Haesse (1971).

4. Calcium (%): Determined using an atomic absorption spectrophotometer.

5. Iron (ppm): Estimated using a Pye Unicam 2900-atomic absorption spectrophotometer (Al-Sahhaf 1989).

RESULTS AND DISCUSSION

The effects of fulvic acid and chitosan on vegetative growth indices

Plant height (cm): The results of the statistical analysis in Table 2 indicated that there were significant differences in the characteristic of plant height when organic fulvic acid was added to the shoot system. The increase was in positive association with the upraised addition level. The increase was in association with the upraised level of the compound addition, as V_4 significantly outperformed all the experimental treatments, exhibiting the highest rate of 108.17 cm, while V_0 displayed the lowest rate (78.83 cm). In the case of spraying the chitosan, C_3 was significantly superior to the control (C_0) and C_1, while there were no significant differences with C_2, exhibiting the highest rate of 99.73 cm (compared to 90.80 cm for C_0). In the case of interaction between the two experimental factors, V_3C_3 was significantly superior to all treatments except for V_4C_3, V_4C_2 and V_4C_1. It achieved the highest rate for the mentioned property (112.00 cm) compared to the comparison treatment which gave the lowest rate (68.00 cm).

Table 2. Effects of fulvic acid and chitosan and their interference in the height of Roselle plants (cm).

Fulvic acid (mL L^{-1})	Chitosan (mL L^{-1})				Effect of Fulvic acid
	C_0	C_1	C_2	C_3	
V_0	68.00	78.67	85.33	83.33	78.83
V_1	86.67	88.00	89.00	88.33	88.00
V_2	93.33	99.67	100.67	104.67	99.58
V_3	99.67	103.67	107.00	112.00	105.58
V_4	106.33	107.67	108.33	110.33	108.17
Effect of Chitosan	90.80	95.53	98.07	99.73	
LSD = 0.05		Fulvic acid	Chitosan	Interference	
		2.214	1.980	4.428	

Leaf area (cm^2)

The results indicated in Table 3 that the addition of humic fulvic acid caused a significant superiority in the leaf area of the Gujarat plants, as V_4 was significantly superior to all treatments except V_3, so that, there were no significant differences between them. The highest rate was recorded at 48.39 cm^2, while V_0 exhibited the lowest rate (37.62 cm^2). The results also indicated that the spraying chitosan had a significant effect on the leaf area, so C_3 exhibited a significant superiority over all the treatments. The highest rate was 46.42 cm^2 compared to 42.76 cm^2 for C_0. The interaction of the two experimental factors had a significant response in the area of the leaf, so V4C3 exhibited a significant superiority over all the interference treatments except for V_3C_3 and V_4C_2, which did not differ significantly and recorded the highest rate (50.34 cm^2), while the lowest rate was found in comparison treatment (35.86 cm^2).

Table 3. Effects of fulvic acid and chitosan and their interference in the leaf area of Roselle plants (cm^2).

Fulvic acid (mL L^{-1})	**Chitosan (mL L^{-1})**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	35.86	38.19	38.19	37.99	37.62
V_1	40.24	44.28	43.28	46.10	43.47
V_2	45.11	45.33	46.53	47.64	46.15
V_3	45.82	47.12	48.23	50.04	47.80
V_4	46.79	47.00	49.41	50.34	48.39
Effect of Chitosan	42.76	44.38	45.18	46.42	
LSD = 0.05		Fulvic acid 0.892	Chitosan 0.798	Interference 1.784	

Number of vegetative branches

The results in Table 4 showed that there were significant differences between the treatments in the number of vegetative branches of Gujarat plants as a result of the addition of humic fulvic acid, and the increase was in association with the upraised level of ground addition. V_4 exhibited the highest rate of 31.33 branches, outperforming all treatments used in the experiment, while V_0 displayed the lowest rate (17.00 branches). The case did not differ from the spraying of the chitosan compound, since C_3 achieved a significant superiority, and this increase was in association with the elevated concentration of chitosan sprayed on the shoot system, achieving the highest rate (26.60 branches), superior to all other treatments. C_0 recorded the lowest rate (22.27 branches). In the case of the interference between the two experimental factors, V_3C_3 exhibited the highest rate (32.33 branches), outperforming all treatments except for V_4C_3, V_4C_2 and V_4C_1. There were no significant differences between them, whereas, the comparison treatment displayed the lowest rate (15.00 branches).

Table 4. Effects of fulvic acid and chitosan and their interference in the number of vegetative branches of Roselle plants (cm^2).

Fulvic acid (mL L^{-1})	**Chitosan (mL L^{-1})**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	15.00	17.00	17.67	18.33	17.00
V_1	19.33	19.00	20.67	22.67	20.42
V_2	22.00	22.67	25.67	27.00	24.33
V_3	25.67	27.67	28.67	32.67	28.67
V_4	29.33	32.00	31.67	32.33	31.33
Effect of Chitosan	22.27	23.67	24.87	26.60	
LSD = 0.05		Fulvic acid 1.614	Chitosan 1.444	Interference 3.228	

The content of the leaf of chlorophyll (SPAD)

The results of the statistical analysis in Table 5 showed that the addition of humic fulvic acid caused a significant response in the chlorophyll content in leaves, and the increase was related to upraised level of ground addition. V_4 exhibited a significant superiority over all other treatments with an average of 50.52 SPAD, while the control group recorded the lowest rate (38.77 SPAD). The results also showed that chitosan had a significant effect on the chlorophyll content of the leaves, so C_3 was significantly superior to C_0, while C_1 did not exhibit significant difference from C_2. It achieved the highest rate of 47.63 SPAD against 44.55 SPAD for C_0. The interference of the experimental factors also displayed a significant effect on the aforementioned character. V_4C_3 exhibited a significant superiority over all the combined treatments without significantly different with V_3C_3, V_4C_2, V_3C_2 and V_4C_1 by achieving the highest rate of 52.13 SPAD in comparison with the control group with the lowest rate (37.27 SPAD).

Table 5. Effects of fulvic acid and chitosan and their interference in chlorophyll leaf content of Roselle plants (SPAD).

Fulvic acid (mL L^{-1})	**Chitosan (mL L^{-1})**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	37.27	39.97	39.49	38.33	38.77
V_1	43.68	44.71	45.11	46.85	45.09
V_2	46.12	47.86	48.13	49.04	47.79
V_3	46.41	47.82	50.37	51.79	49.10
V_4	49.29	49.98	50.69	52.13	50.52
Effect of Chitosan	44.55	46.07	46.76	47.63	
LSD = 0.05		Fulvic acid 1.360	Chitosan 1.216	Interference 2.720	

Dry weight of the shoot system (g)

The results of the statistical analysis in Table 6 indicated that the addition of fulvic acid had a significant effect on the dry weight of Roselle plants, and the increase was in association with the upraised level of ground addition of humic acid. So that, V_4 exhibited a significant superiority over all the experiment treatments, revealing the highest rate of 546.1 g compared to 342.8 g for V_0. Chitosan also contributed to a significant increase in the above character, so C_3 achieved the highest rate of 482.5 g, outperforming all treatments, while C_0 displayed the lowest rate (412.4 g). The interference of the two factors exhibited a significant effect on the dry weight of plants. So that, V_4C_3 achieved a significant superiority over all the treatments except for V_4C_2, V_3C_3, V_4C_1 and V_3C_2, as there were no significant differences between them, so it achieved an average of 572.4 g, in comparison with the control, recording the lowest rate (280.1 g).

Table 6. Effects of fulvic acid and chitosan and their interference in dry weight of shoot system of Roselle plants (g).

Fulvic acid (mL L^{-1})	**Chitosan (mL L^{-1})**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	280.1	357.2	350.2	383.5	342.8
V_1	390.7	406.4	443.5	440.5	420.3
V_2	451.8	461.9	460.4	457.5	457.9
V_3	446.8	521.8	538.8	558.6	516.5
V_4	492.7	555.9	563.2	572.4	546.5
Effect of Chitosan	412.4	460.7	471.2	582.5	
LSD = 0.05		Folvlic acid 17.14	Chitosan 15.33	Interference 34.28	

Effects of fulvic acid and chitosan on the leaf nutrient contents

Nitrogen content of leaves (%)

The results of Table 7 showed that there were significant differences between the treatments resulting from the ground addition of humic fulvic acid in the nitrogen content of the leaves, as the increase was in association with the upraised level of the acid addition. V_4 exhibited a significant superiority over all treatments except for V_3, which did not differ significantly with each other.

It exhibited the highest rate of 1.405%, while V_0 the lowest rate (1.173%). In addition, spraying chitosan on the shoot system resulted in a significant response in the aforementioned character. C_3 displayed the highest rate of 1.390%, superior to all treatments except for C_2, which did not differ significantly. C_0 exhibited the lowest rate of 1.228%. The interference of the two factors also exhibited a significant effect on the nitrogen content of the leaves, so V_3C_3 achieved a significant superiority over all the treatments except for V_4C_3, V_3C_3 and V_3C_2, with no significant differences between them. So the highest rate was recorded at 1.487% in comparison with the control group, which exhibited the lowest rate (1.010%).

Table 7. Effects of fulvic acid and chitosan and their combination in the leaf nitrogen content of Roselle plants (%).

Fulvic acid ($mL\ L^{-1}$)	**Chitosan ($mL\ L^{-1}$)**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	1.010	1.227	1.203	1.253	1.173
V_1	1.277	1.287	1.317	1.347	1.307
V_2	1.217	1.340	1.390	1.387	1.333
V_3	1.297	1.377	1.457	1.487	1.404
V_4	1.340	1.403	1.477	1.477	1.405
Effect of Chitosan	1.228	1.327	1.353	1.390	
LSD = 0.05		Fulvic acid 0.041	Chitosan 0.037	Interference 0.083	

Phosphorous content of the leaves (%)

The results in Table 8 showed that the addition of humic fulvic acid caused a significant response in the phosphorous content of the leaves. V_3 exhibited a significant superiority over the control (V_0) and V_1. It did not differ significantly from V_4 and V_2 with a rate of 0.268%, while V_0 displayed the lowest rate (0.218%). The results also showed that chitosan had a significant effect on the phosphorous content of leaves, so C_3 exhibited a significant superiority over C_0. In addition, C_1 only, did not differ significantly with C_2, which was recorded the highest rate of 0.267% in comparison with 0.219% for C_0. The interference of the two experimental factors also exhibited a significant effect, so V_2C_3 achieved a significant superiority in this character without significantly different with many of the interference coefficients by displaying the highest rate of 0.287% compared to the control treatment with the lowest rate (0.193%).

Table 8. Effects of fulvic acid and chitosan and their interference in the leaf phosphorus content of Roselle plants (%).

Fulvic acid ($mL\ L^{-1}$)	**Chitosan ($mL\ L^{-1}$)**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	0.193	0.220	0.217	0.240	0.218
V_1	0.210	0.243	0.243	0.250	0.237
V_2	0.217	0.257	0.277	0.287	0.259
V_3	0.240	0.273	0.280	0.277	0.268
V_4	0.237	0.277	0.263	0.283	0.265
Effect of Chitosan	0.219	0.254	0.256	0.267	
LSD = 0.05		Fulvic acid 0.012	Chitosan 0.011	Interference 0.025	

Potassium content of leaves (%)

It is clear from the results of Table 9 that there were significant differences in the potassium content of leaves when adding fulvic humic acid to the field soil, and the increase was in association with the upraised level of the acid added. V_4 was significantly superior to all treatments except for V_3, which did not differ significantly from each other. It achieved the highest rate of 1.853%, while the control (V_0) achieved the lowest (1.707%). In the case of spraying the chitosan compound, C_3 was significantly superior to C_0 and C_1 only. It did not differ significantly from C_2, exhibiting the highest rate of 1.848% compared to 1.682% for C_0. In the case of the interference of the two factors, V_3C_3 exhibited a significant superiority without significantly different with many of the other treatments. So it achieved the highest rate (1.920%) compared to the control, with the lowest rate of 1.620%.

Table 9. Effects of fulvic acid and chitosan and their interference in the content of Roselle plants from potassium (%).

Fulvic acid (mL L^{-1})	**Chitosan (mL L^{-1})**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	1.620	1.703	1.740	1.763	1.707
V_1	1.570	1.763	1.803	1.797	1.733
V_2	1.727	1.810	1.823	1.850	1.801
V_3	1.737	1.840	1.867	1.920	1.841
V_4	1.757	1.863	1.883	1.910	1.853
Effect of Chitosan	1.682	1.796	1.823	1.848	
LSD = 0.05		Fulvic acid 0.049	Chitosan 0.044	Interference 0.097	

Calcium content of leaves (%)

The results of the statistical analysis in Table 10 indicated that the addition of humic fulvic acid caused a significant superiority in the calcium content of the leaves of Roselle plants. So that, V_4 was significantly superior to all treatments except for V_3 with no significant differences with each other, recording the highest rate of 3.811%. The control (V_0) exhibited the lowest rate of 3.567%. The results also showed that chitosan had a significant effect on the aforementioned character. C_2 achieved a significant superiority over all treatments except for C_3, since they did not differ significantly by recording the highest rate of 3.779% compared to 3.503% for C_0. The interference of the two experimental factors exhibited a significant increase in the calcium content of leaves. So, V_4C_2 exhibited a significant superiority over most of the interfered treatments, recording the highest rate of 3.913%, while the lowest in the control (3.313%).

Table 10. Effects of fulvic acid and chitosan and their interference in the leaf calcium content of Roselle plants of (%).

Fulvic acid (mL L^{-1})	**Chitosan (mL L^{-1})**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	3.313	3.610	3.697	3.650	3.567
V_1	3.413	3.657	3.690	3.663	3.606
V_2	3.563	3.610	3.723	3.750	3.662
V_3	3.553	3.770	3.913	3.887	3.781
V_4	3.673	3.820	3.870	3.880	3.811
Effect of Chitosan	3.503	3.693	3.779	3.766	
LSD = 0.05		Fulvic acid 0.079	Chitosan 0.071	Interference 0.158	

Iron content of leaves (ppm)

The results of Table 11 showed that there were significant differences between the treatments in the iron content of the Roselle plants leaves due to the addition of humic fulvic acid to soil. It outperformed than all treatments used in the experiment without significant difference with V_3, while V_0 exhibited the lowest rate (51.52 ppm). The case was not different for spraying chitosan. C_3 displayed a significant superiority over all treatments, and the increase was in association with the chitosan concentration used as a spray. On the shoot system, it achieved the highest rate of 65.58 ppm, while C_0 recording the lowest (55.11 ppm). The results of Table 11 showed significant differences between the coefficients of the iron component of the Gujarat plants due to the terrestrial addition of follicular acid. The increase was positively associated with the added level, so V_4 exhibited the highest rate of 65.85 ppm. The coefficients used in the experiment were not significantly different from V_3, while V_0 displayed the lowest rate (51.52 ppm). The Chitosan treatments did not exhibit significant difference. C_3 was significantly higher in all of the above treatments. The increase was related to the level of chitosan used on the vegetative group, exhibiting the highest rate of 65.58 ppm, while C_0 recorded the lowest rate (55.11 ppm). In the case of interference between the two experimental factors, V_4C_3 recorded the highest rate of 69.88 ppm, superior to all treatments except for V_4C_3, V_4C_2 and V_3C_2, with no significant differences between them. The control treatment exhibited the lowest average (43.16 ppm).

Table 11. Effect of fulvic acid and chitosan and their interference in the leaf content of Roselle plants of iron element (ppm).

Fulvic acid (mL L^{-1})	**Chitosan (mL L^{-1})**				**Effect of Fulvic acid**
	C_0	C_1	C_2	C_3	
V_0	43.16	47.51	56.62	58.81	51.52
V_1	53.55	61.46	62.75	63.87	60.41
V_2	58.57	61.57	65.75	65.82	62.76
V_3	59.57	65.61	68.32	69.53	65.76
V_4	60.72	66.18	66.63	69.88	65.85
Effect of Chitosan	55.11	60.47	63.88	65.58	
LSD = 0.05		Fulvic acid 1.643	Chitosan 1.469	Interference 3.286	

DISCUSSION

The significant increase achieved in the vegetative growth properties of Roselle plants as a result of the soil addition of fulvic humic acid and the treatment of the shoot system by spraying with chitosan compound may be due to the role of both compounds in improving growth according to the conditions of the experiment, since the increase was in association with upraising the level of treatments with both compounds for most of the examined characters. The significant increase in vegetative growth indices as a result of treating plants with organic fulvic acid could be explained by its role in the elevated permeability of cell membranes, which contributes and accelerates the absorption of nutrients through the leaves and their transfer to the different parts of the plant followed by their accumulation in the final estuaries (Sink). In addition, it plays a role in the reduced food competition between plant parts, hence, the increased essential nutrient contents of the plant parts and elevating the readiness of the necessary nutrients, which upraises the dry matter in the plant. This is reflected in the elevated plant height, dry weight and the number of vegetative branches formed (El-Desuki *et al.* 2010). In addition to its role in improving the chemical and physical properties of the soil, enhancing its moisture content, improving aeration, and preventing the exposure of nutrients to washing by irrigation water, which increases the cation exchange capacity (Taj Eldin and Al-Barakat, 2013a). It also has the ability to make the soil dark in colour, which helps in absorbing sunlight significantly, thus raising the temperature of the soil, and this contributes to warming the root system on the one hand and stimulating its growth and increasing its branches on the other hand. In addition to its important role in preventing the cracking of the surface of the soil, this leads to preventing the cutting of the developing root hairs. This can elevate the transverse area of the leaf as a result of the accumulation of absorbed nutrients. At the same time, it can upraise the leaves chlorophyll contents due to the ability of the roots to absorb the ready magnesium in the soil (Halvin *et al.* 2005). Al-Jumaili (2012) also indicated that fulvic acid plays an important role in enhancing the biological properties of soil by elevating the biological activity of beneficial microorganisms in the soil due to its role in the increased aeration and the reduced toxicity of substances in the soil, which leads to activating their work.

The obtained results were in agreement with those obtained by Majul *et al.* (2014) and Sami *et al.* (2015), since the humic acid exhibited a significant effect on the increased vegetative growth indices of Roselle plants. The results of the current study also were in line with the results of Taj Eldin & Al-Barakat (2016b). The addition of humic fulvic acid at several levels to the soil of the field planted with *Zea mays* contributed to a significant increase in the vegetative growth indices, which was in association with the upraised level of the compound additions. Taj Eldin & al-Barakat (2016a) mentioned that the significant elevation in the percentage of nitrogen in leaves may be due to considering that the fulvic humic acid addition is a storehouse of nutrients, including nitrogen, as well as its role in improving soil properties, along with the upraised readiness of nutrients absorbable by the roots, or because the humic acid solution contains nitrogen in its composition (Table 1). In the case of the increased percentage of phosphorous, it can be attributed to the role of acid in reducing the processes of deposition and adsorption of phosphorus on the surfaces of colloids as a result of competition for adsorption sites. This increases the release of phosphorus into the soil as well as its slow and continuous dissolution due to the addition of fulvic acid (Abdel - Razzak & Al-Sharkawy 2013). Fulvic acid plays an important role in increasing the absorption of monovalent ions such as ammonium and potassium by activating the uptake of roots, which is reflected in the accumulation of potassium in different parts of the plant, and this may explain the significant elevation of potassium in leaves (Shahryari *et al.* 2011), or according to Al-Jumaili (2012), fulvic acid plays a role in the upraised availability of potassium in the soil due to its lack of fixation as a result of substituting the H^+ ion resulting from the dissolution of organic acids with the K^+ ion on the exchange surfaces.

The significant increase in the calcium content of leaves may be due to the content of the nutrient solution enhanced with an amount of calcium (Table 1) and this helps the increased amount of calcium inside the plant, or according to Jassem & Al-Dulaimi (2014) fulvic acid has the ability to treat the salinity of the field soil by chelating the calcium present in the soil. Then, this element becomes free, active and easily absorbed by the root system. In the case of the significant iron increase as a result of treatment with fulvic humic acid, this may be due to its role in facilitating the absorption of iron from the soil, since the acid chelates the iron element available in the soil as iron oxides, making it ready for absorption (Abdolkarim 2012). In the case of the effect of the chitosan on plant growth and the increased nutrient contents, Amin (2013) reported that the chitosan exhibits a very important effect on the upraised metabolic processes inside the plant, and consequently, the increased accumulation of dry matter inside it, as well as accelerating the growth and development of the plant during the period of its life cycle. It is also important in elevating the support of the plant, since it is a fibre extracted from chitin, and their chemical composition is similar to each other. Chitosan enters the composition of the cell walls and elevates the strength of cells as well as giving them strength and immunity against the various stress conditions of the plant. It also acts as an antioxidant against harmful free radicals in the plant (Salman & Al-Abadi 2009). Chitosan also possesses three effective functional groups, the amine group and the primary and secondary carboxyl groups on the sites of the second, third and sixth carbon atoms, respectively, and this in turn is of great importance to plants (Speiciene *et al.* 2007). Hence, according to the experiment results, the chitosan may play a direct role in elevating the growth indices.

REFERENCES

Abbas, HAH 2016, The effect of some natural control parameters before and after the harvest on improving the qualitative characteristics and susceptibility of the date palm fruit *Phienix dactylifera* L. Sanchis Al-Barhi and Al-Braim. PhD Dissertation, College of Agriculture, University of Basra. Iraq.

Abdel Razzak, HS & El Sharkawy, GA 2013, Effect of bio-fertilizer and humic acid applications on growth, yield, quality and storability of tow garlic (*Allium Sativum* L.) Cultivars. *Asian Journal of Crop Science*, 5: 48-64.

Abdolkarim, K 2012, Study effects of biological, manure and chemicals nitrogen fertilizer application under irrigation management in lentil farming on physiochemical properties of soil. *Journal of Basic and Applied Scientific Research*, 2: 6483-6487.

Ali, NS 2012, Fertilizer technologies and uses. University House for Printing, Publishing and Translation. Baghdad University. Ministry of Higher Education and Scientific Research, Iraq.

Al Jumaili, MOS 2012, The combined effect of spraying with humic acid (humic and fulvic acid) and potassium fertilization method on the growth and yield of potatoes. PhD Dissertation, College of Agriculture, University of Baghdad, Baghdad, Iraq.

Al Nuaimi, SANA 1999, Fertilizers and soil fertility. House of Books for Printing and Publishing, University of Al Mosul. Ministry of Higher Education and Scientific Research, Baghdad, Iraq.

Al Sahaf, FH 1989, Applied plant nutrition. Ministry of Higher Education and Scientific Research, Book House Press, University of Mosul, Mosul, Iraq.

Al Sahuki, MM & Waheeb, Karima 1990, Applications in the design and analysis of agricultural experiments. Dar Al Hekma Printing Press. Ministry of Higher Education and Scientific Research, Baghdad University, Baghdad, Iraq.

Al Tahafy, Sami AAM, Hussein, AS, Habib, HA & Azab, NH 2015, Response of growth and yield of honeysuckle plant (Hibiscus sabdariffa L.) to the addition of a clean salt and humic aljohara in highly saline soils. *Kufa Journal of Agricultural Sciences*, 7: 73 - 93.

Amin, MM 2013, Chitosan. *Journal of Environmental Studies,* 38: 1-8.

Azooz, MM 2009, Foliar application with riboflavin (vitamin B_2) enhancing the resistance of *Hibiscus sabdariffa* L. to Salinity stress. *Journal of Biological Sciences*, 9: 109-118.

Bala, MG & Fagbayide, JA 2009, Effect of nitrogen fertilizer on the growth and calyx yield of two cultivars of Roselle in Northern Guinea Savanna. *Middle-East Journal of Scientific Research*, 4: 66-71.

Devlieghere, F, Vermeulen, A & Debevere, J 2004, Chitosan: antimicrobial activity, interaction with food components and applicability as a coating on fruit and vegetables. *Food Microbiology*, 21: 703.

El Desuki, M, Hafez, MM, Mahmoud, AR & Abd Albaky, FS 2010, Effect of organic and bio-fertilizers on the plant growth, green pod yield , quality of pea. *International Journal of Academic Research*, 2: 87-92.

Eldin, T, Majid, M & Al Barakat, HNK 2016a, The effect of bio-fertilization, foliar spray, and ground addition of humic and fulvic acids on N, P, and K readiness in soil. *Al-Muthanna University Journal for Agricultural Sciences*, 4: 56 - 68.

Eldin, T, Majid, M & Al Barakat, HNK 2016a, The effect of bio-fertilization, foliar spray, and ground addition of humic and fulvic acid on the growth and yield of maize (*Zea mays* L.). *Al-Muthanna University Journal for Agricultural Sciences*, 4: 75 - 68.

Ghasemnezhad, M, Shiri, MA & M. Sanavi, M 2010, Effect of chitosan coatings on some quality indices of apricot (*Prunus armeniaca* L.) during cold storage, *Caspian Journal of Environmental Sciences*, 8: 25-33.

Galaudu, MS 2006, Effect of moisture on the germination rate of Roselle plant. Proceeding of 23rd Annual Conference. Horticultural Science of Nigerian, pp: 30-32.

Gresser , MS & Parsons JM 1979, Sulfuric-perchloric acid digestion of some nutrients .*Egyptian Journal of Horticulture*, 25: 55-70.

Hassoun, AS 2017, The effect of salicylic acid and marine algae extract on the growth and yield of several varieties of cactus plant and its content of some active medicinal compounds. MSc. Dissertation, Al Furat Central Technical University, Technical College, Al Musayyib, Iraq.

Havlin, JL, Beaton, JD, Tisdal, SL & Nelson, WL 2005, Soil fertility and fertilizers, an introduction to nutrient management. 7th ed., Upper Saddle River New Jersey. USA, 515 p.

Imran, BM 1988, The Aljarat plant cultivation and medical nutritional benefits. *Journal of Medicine and Science*, 15: 16.

Jasem, AH & Al Dulaimi, QL 2014, The effect of adding organic fertilizers, humus acid and seaweed extract on the growth and yield of green pods of peas (*Vicia faba* L.), *Al Furat Journal of Agricultural Sciences*, 6: 163-172.

John, MK 1970, Calorimetric determination of phosphorus in soil and plant materials with ascorbic acid soil Science, 109: 214-220.

Majoul, AK & Hamza, KK & Kassar, HI 2012, The effect of foliar spray of organic fertilizer humic acid and planting distances on growth and yield of Hibiscus sabdariffa L., *Al-Qadisiya Journal of Agricultural Sciences*, 2: 40-46.

Rao, PU 1996, Nutrient composition and biological evaluation of Mesta (*Hisbicuss abdariffa* L.). *Seeds Plant Foods for Human Nutrition*, 49: 27-34.

Rostamzad, H, Abbasi Mesrdashti, R, Akbari Nargesi, E & Fakouri, Z 2019, Shelf life of refrigerated silver carp, Hypophthalmichthys molitrix, fillets treated with chitosan film and coating incorporated with ginger extract. *Caspian Journal of Environmental Sciences*, 17: 143-153

Salman, D & Al Abadi IMK 2009, Evaluation of some physiochemical and functional properties of ketosan prepared with an alkaline treatment of shrimp crusts. *Iraqi Journal of Agricultural Sciences*, 40: 63-75.

Shahryari, R, Khayatnezhad, M & Bahari, N 2011, Effect of two humic fertilizers on germination and seedling matter and a proposed modification of the chromic acid titration method. *Soil Science*, 34: 29-38.

Speiciene, V, Guilmineau, FU & Kulazik, LD 2007, The effect of chitosan on the properties of emulsions stabilized by whey proteins. *Food Chemistry*, 102: 1048-1054.

Zahmatkesh, A, Karimzadeh, K, Faridnia, M 2020, Effect of dietary selenium nanoparticles and chitosan oligosaccharide on biochemical parameters of Caspian roach (*Rutilus caspicus*) under malathion stress. *Caspian Journal of Environmental Sciences*, 18: 59-71.

Bibliographic information of this paper for citing:

Asree, H,J, Kareem, N,E, Khirallah, A,A 2022, Effect of fulvic acid and chitosan in the growth of Roselle plants, *Hibiscus sabdarrifa* L and their nutrient content. Caspian Journal of Environmental Sciences, 20: 373-383.