

## Effect of Organic Fertilization and Seaweed Extract on Growth, Yield, and Bioactive Compounds of Roselle Plant (*Hibiscus sabdariffa L*)

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### Abstract

*A field experiment was conducted during the spring season of 2025 at a private farm in Al-Jilawiya village, Al-Musayyib district, located about 40 km north of Babil province, Iraq, to evaluate the effect of seaweed extract and animal organic fertilizer on growth, yield, and anthocyanin content of roselle (*Hibiscus sabdariffa L.*). The experiment was arranged as a factorial experiment in a Randomized Complete Block Design (RCBD) with three replications. The study included three factors: two roselle cultivars (red and purple), three concentrations of seaweed extract (0, 3, and 6 ml L<sup>-1</sup>), and three levels of animal organic fertilizer (cow manure) (0, 3, and 6 ton ha<sup>-1</sup>).*

*The results showed that the purple cultivar significantly outperformed the local red cultivar in plant height, number of main branches, number of capsules per plant, calyx yield per plant, and anthocyanin content, recording mean values of 64.18 cm, 5.16 branches plant<sup>-1</sup>, 141.54 capsules plant<sup>-1</sup>, 46.31 g plant<sup>-1</sup>, and 0.284%, respectively. The application of seaweed extract at 6 ml L<sup>-1</sup> resulted in the highest averages for the studied traits, reaching 169.64 cm for plant height, 5.40 branches plant<sup>-1</sup>, 163.31 capsules plant<sup>-1</sup>, 48.13 g plant<sup>-1</sup> for calyx yield, and 0.317% anthocyanin content. Similarly, the highest level of organic fertilizer (6 ton ha<sup>-1</sup>) significantly improved plant growth and productivity, producing the highest mean values of 164.68 cm plant height, 5.47 branches plant<sup>-1</sup>, 164.44 capsules plant<sup>-1</sup>, 50.51 g plant<sup>-1</sup> calyx yield, and 0.347% anthocyanin content.*

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*These findings indicate that the combined use of seaweed extract and organic fertilizer can effectively enhance growth, yield, and bioactive compound content of roselle plants under the environmental conditions of central Iraq.*

## **Introduction**

Hibiscus sabdariffa L., commonly known as roselle, belongs to the family Malvaceae. It is considered an annual medicinal plant. Its original habitat is not clearly known, but it is believed to originate from the tropical and subtropical regions of Africa, while some taxonomists suggest that its original homeland is India (Al-Humaidi, 2023). Its main medicinal importance lies in its calyces, which are a source of glycosides and Hibiscin. These calyces are rich in vitamin C and organic acids such as citric, tartaric, and malic acids. They also contain the compound protocatechuic acid (PAC), which is considered an effective antioxidant and plays a significant role in combating certain cancerous tumors (Ajithadoss et al., 2017).

This compound is regarded as a rich source of strong antioxidant substances that help the body resist free radicals and enhance the immune system. As a result, it helps protect against many diseases and health problems such as joint and bone inflammation, heart diseases, cancer, and premature aging, in addition to many other conditions. Besides its medicinal uses, it is also used as a refreshing beverage to reduce high body temperature. The red pigment extracted from it is industrially used as a natural food colorant and flavoring agent because it is involved in the production of jams, jelly, and confectionery. Its seeds also contain a high percentage of oil ranging between 20–25% (Mohammed Hassan, 2025; Louis et al., 2017).

Organic fertilizers added to the soil are considered less harmful to the environment compared to chemical fertilizers. They release nutrients gradually because their decomposition occurs slowly through microorganisms that convert organic materials into mineral elements ready for plant absorption. This process positively affects the growth and yield of roselle plants (Al-Rome, 2014).

Due to the importance of plants, it has become necessary to increase the productivity of their active compounds. Therefore, efforts must be made to improve both the quantity and quality of production through methods such as foliar nutrition, including the use of seaweed extracts. These extracts are considered an important nutritional source for crops and have recently been used because they reduce the need for chemical fertilizers. Practically, they have shown a clear effect in improving both the quantity and quality of production. They are also a good source of nutrients and many important growth stimulants such as auxins and cytokinins, in addition to their content of organic and amino acids and certain vitamins that influence vital plant activities (Al-Ajili, 2024).

### **Objectives of the Study**

1. To determine the superior cultivar that produces the highest averages for growth traits, yield, and medically active compounds.
2. To determine the best concentration of seaweed extract that achieves the highest averages for the studied traits.
3. To identify the optimal level of organic fertilizers that provides the highest averages for growth, yield, and active compounds.
4. To identify the superior interactions (two-way and three-way) among the studied factors for all the investigated traits.

### **3. Materials and Methods**

#### **3.1 Experimental Site**

A field experiment was conducted in Al-Jilawiya area, Al-Musayyib District, during the spring growing season of 2025 at geographical coordinates 32.690302° N latitude and 44.418786° E longitude. The study aimed to evaluate the effects of organic fertilization and seaweed extract on the growth and yield performance of two cultivars of roselle (*Hibiscus sabdariffa* L.).

Seeds of the two cultivars were obtained from the Roselle Tea Development Project in Al-Diwaniyah, which is affiliated with the Directorate of Agriculture in Al-Diwaniyah, Iraq.

### 3.2 Soil Sampling and Analysis

Prior to planting, five soil samples were randomly collected from the experimental field at a depth of 0–30 cm. The collected samples were cleared of debris, thoroughly mixed to obtain a composite sample, air-dried, and homogenized. A representative subsample was then subjected to laboratory analysis to determine selected physical and chemical soil properties, following the procedures described by Page et al. (2000).

The soil analyses were conducted in the Soil Laboratory of Al-Musayyib Technical Institute.

Table 1. Selected physical and chemical properties of the experimental soil

Unit of Measurement	Quantity	Attribute
-----	7.1	Soil reactivity level (PH)
Ds.m-1	3.4	Electrical conductivity (CE)
mg.kg-1	33.1	Total nitrogen
mg.kg-1	29	Ready-made phosphorus
mg.kg-1	22-143	Ready-made potassium
gm.kg-1	4.3	organic matter
g.kg-1	466	sand
g.kg-1	387	silt
g.kg-1	147	clay
	silty sand mixture	weave

### 3.3 Soil Preparation

The experimental field was initially plowed using a moldboard plow, followed by harrowing and leveling to ensure suitable soil conditions for planting. The field was then divided into three replicates, each consisting of 18 experimental plots.

The area of each experimental plot was 6 m<sup>2</sup> (3 m × 2 m) and included four ridges. The spacing between ridges was 75 cm, while the distance between planting holes was 50 cm.

Two roselle cultivars (red and purple) were sown on 21 April 2025, with 2–3 seeds per planting hole. The field was irrigated immediately after sowing. When plants reached approximately 15 cm in height, thinning and gap filling were performed to maintain uniform plant density.

Throughout the growing season, standard agronomic practices including irrigation, hoeing, weeding, and pest control were applied as required to maintain optimal crop growth.

### 3.4 Experimental Design and Treatment Factors

The experiment was conducted using a Randomized Complete Block Design (RCBD) with a factorial arrangement and three replicates. The study included three experimental factors:

First: Two varieties of kojrat (red and eggplant), designated (v1, v2) respectively.

Second: Organic fertilization at concentrations of 0, 3, and 6 tons ha<sup>-1</sup>, designated (M0, M1, M2) respectively.

Third: Seaweed extract at concentrations of 0, 3, and 6 ml L<sup>-1</sup>, designated (A0, A1, A2) respectively.

#### Organic Fertilizer Application

Fermented cattle manure was used as the organic fertilizer source. The manure was incorporated into the soil during land preparation on 21 March 2025, approximately one month prior to sowing. The fertilizer was applied according to the experimental treatments at rates of 0, 3, and 6 ton ha<sup>-1</sup> for each experimental plot.

#### Seaweed Extract (Tecamin Algae)

The seaweed extract (Tecamin Algae) is a natural concentrated bio-fertilizer derived from green algae collected from aquatic environments and subsequently dried and processed. The extract contains essential nutrients, trace elements, vitamins, amino acids, enzymes, plant growth regulators, and carbohydrates, which contribute to improved plant growth and productivity.

**Table 2. Chemical composition of seaweed extract**

ratio	Material
16%	Dried seaweed
13%	Organic matter
1%	Total nitrogen
0.50%	Total phosphorus
2%	Total potassium
1.50%	Amino acids

The extract was applied as a foliar spray at concentrations of 0, 3, and 6 g L<sup>-1</sup> until complete wetting using a 16-L knapsack sprayer. A total of three spraying applications were performed during the plant growth period until maturity.

#### **Vegetative Growth Characteristics:**

Six plants were randomly selected from each experimental unit to study the following characteristics:

##### **1.Plant Height (cm)**

Plants were randomly selected from each experimental unit. Plant height was measured using a metric tape measure from the soil surface to the uppermost internode, and the average plant height was calculated.

##### **2. Number of Main Branches (Branch/Plant – 1)**

The number of main branches was counted for the randomly selected plants, and the average was calculated.

#### **Yield characteristics and components**

1. Number of capsules (capsules per plant-1)

The number of capsules per plant was calculated from plants randomly selected from each experimental unit, and the average was then calculated.

2. Yield per plant of sepals for dry weight (g. plant – 1)

Measure the dry weight of the sepals by collecting tender sepals and air-drying them in the shade to prevent volatilization of the active ingredient for three weeks until the weight stabilizes (g. plant - 1).

**Properties of active and medicinal substances**

**1.Estimation of the anthocyanin content of the calyx leaves:**

Anthocyanins were estimated using the pH differential method, according to AlHussainy's (2010) method. A buffer solution (KCl, 0.25M, pH=1.0) was prepared by dissolving 1.86 g of KCl in 980 ml of distilled water. The pH was then adjusted to 1.0 using concentrated HCl, and the volume was completed with distilled water to 1 liter. The buffer solution (CH<sub>3</sub>COONa, 0.4M, pH 4.5) was prepared by dissolving 54.43 g of CH<sub>3</sub>COONa in 960 mL of distilled water. The pH was adjusted to 4.5 using HCl, and the volume was then brought up to 1 L using distilled water. One g of anthocyanin extract was dissolved in 10 mL of acidified methanol (85 mL methanol + 15 mL HCl). 0.8 mL of this solution was withdrawn, and the volume was brought up to 3 mL with a potassium chloride buffer solution (KCl, 0.025 M, pH 1.0). The same step was repeated, bringing the volume up to 3 mL with a sodium acetate buffer solution (CH<sub>3</sub>COONa, 0.4M, pH 4.5). After 15 minutes, the absorbance of the two samples was measured at 520 and 700 nm. The same steps were performed using Methanol and acidified water served as the blank sample. The anthocyanin content (mg/L) was estimated using the following equations:

$$A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH 1}} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH 4.5}} \dots\dots(1)$$

$$\text{Anthocyanin content (mg/L)} = A \cdot \text{MW} \cdot \text{DF} 1000 / \dots\dots(2)$$

Therefore:

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=DF = Dilution factor

=L = Path length (1 cm)

$\varepsilon$  = Absorption coefficient (L/mol.cm 26900) Cyandine-3-glucoside

MW = Molecular weight of Cyandine-3-glucoside = 449.2 g/mol

### Statistical Analysis

The data were statistically analyzed for the factorial experiment according to the design of completely randomized blocks using the (Genstat) program and using the Least Significant Difference (LSD) test to compare means at a probability degree of (0.05) genstat (2017).

### Plant Height Trait

The table shows that the eggplant variety (V2) significantly outperformed the red variety (V1), exhibiting the highest average plant height of 64.18 cm, while the red variety (V1) yielded the lowest average height of 128.8 cm. This represents an increase of 21.54 cm in plant height. The difference in height between the varieties is attributed to variations in their genetic makeup. Additionally, there are differences in the physiology of each plant, as well as variations in its environmental influence. Environmental factors play a significant role in influencing the varieties' height, resulting in different readings for various traits, including plant height.

Regarding the seaweed extract, concentration S2 outperformed the other concentrations, yielding the highest average height of 169.64 cm. Concentration S0 (the control treatment) produced the lowest average height of 125.06 cm. This represents an increase in height, as measured by the ratio of the two seaweed extract concentrations. The marine algae concentration reached 26.27%, and the same results were obtained: concentration S2 gave the highest average height for the studied trait (plant height), reaching 169.64 cm, while

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concentration S0 gave the lowest average height of 125.06 cm. This is because marine algae contain both macro and micronutrients, resulting in a 26.27% increase in the trait.

This is because the extract contains nitrogen, which aids in the synthesis of prophyrin, a component of the chlorophyll molecule. Prophyrin also accelerates cell division, particularly in the stem, leading to its elongation (Sadak, 2018).

However, when animal manure (cow dung) was added, the O2 treatment outperformed others, yielding the highest average height for the studied trait (plant height) at 164.68 cm, while the control treatment O0 yielded the lowest average at 120.19 cm. This resulted in a 27.01% increase in the trait. This can be explained by the fact that cow manure contains living organisms. When these organisms decompose, they release nutrients in compounds that the plant can easily absorb, such as nitrates, thus increasing stem cell division and resulting in increased height.

As for the dual interaction between the variety and organic fertilization, the combination (O2×V2) was superior, yielding the highest average height at 209.50 cm, while the combination (O0×V1) yielded the lowest average height at 112.15 cm. This resulted in a 46.46% increase in the trait. In the dual interaction between the two varieties and the seaweed extract, the combination (S2×V2) yielded the highest average length of 191.16 cm, while the combination (S0×V1) yielded the lowest average length of 110.59 cm. This resulted in a 42.14% increase in the trait.

In the dual interaction between organic fertilizer and another seaweed extract, the combination (S2×O2) yielded the highest average length of 168.96 cm, while the combination (S0×O0) yielded the lowest average length of 100.84 cm. This resulted in a 40.31% increase in the trait.

As for the tripartite interaction between the three experimental factors (varieties × organic fertilizers × seaweed extract), the combination (S2×O2×V2) was superior, giving the highest average for the trait, which reached 247.10 cm, while the combination (S0×O0×V1) gave the lowest average for the trait, which reached 90.27 cm. Thus, an increase in the trait of 63.46% was obtained.

Average variety × organic fertilization	seaweed extract			organic fertilizer	Categories
	S2	S1	S0		
112.15	133.60	112.57	90.27	O0	V1 red
128.58	142.49	127.69	115.57	O1	
145.70	168.28	142.89	125.93	O2	
128.23	144.68	128.60	111.41	O0	V2 eggplant
154.84	181.70	152.45	130.37	O1	
209.50	247.10	204.60	176.79	O2	
	169.64	144.80	125.06		average
3.376	5.847		2.387		L.S.D

Average varieties	S2	S1	S0	Algae Varieties
128.80	148.12	127.71	110.59	red V1
164.18	191.16	161.88	139.52	eggplant V2
	169.64	144.80	125.06	average
1.914	3.476			L.S.D

Average organic fertilizer	S2	S1	S0	Algae Organic fertilizer
120.19	139.14	120.58	100.84	O0
141.71	162.10	140.07	122.97	O1
164.68	168.96	173.74	151.36	O2
	156.73	144.80	125.06	average
2.387	4.135			L.S.D

### Number of Main Branches

The attached tables show semi-semi ... Regarding the effect of organic fertilizer (cow manure), the O2 treatment was superior, yielding the highest average for the trait at 5.47 branches per plant, while the O0 (control) treatment yielded the lowest average at 4.47 branches per plant. This resulted in an 18.20% increase in the trait. The increase in the number of branches per plant in the crops treated with organic fertilization can be attributed to the nitrogen content of organic fertilizer, which in turn promotes cell division and vegetative growth. This, in turn, stimulates photosynthesis, leading to an increase in most vegetative traits. Since the number of branches is a vegetative trait, it increases accordingly.

As for the dual interaction of variety ratio and organic fertilization, the (O2×V2) combination was superior, yielding the highest average for the trait at 5.82 branches per plant, while the (O0×V1) combination yielded the lowest average at 4.53 branches per plant. In the binary interaction of the variety and seaweed extract, the combination (S2×V2) yielded the highest average of 5.71 plant branches per 1000, while the combination (S0×V1) yielded the lowest average of 4.37 plant branches per 1000. Similarly, in the binary interaction of the organic fertilizer and seaweed extract, the combination (S2×O2) yielded the highest average of 5.99 plant branches per 1000, while the combination (S0×O0) yielded the lowest average of 4.19 plant branches per 1000.

In the ternary interaction of the variety, organic fertilizer, and seaweed extract, the combination (S2×O2×V2) yielded the highest average of 6.44 plant branches per 1000, while the combination (S0×O0×V1) yielded the lowest average of 4.09 plant branches per 1000.

Varieties *Organic Fertilization	Seaweed extract			Organic fertilization	Varieties
	S2	S1	S0		
4.53	4.64	4.37	4.09	O0	V1 red
4.68	5.10	4.66	4.29	O1	
5.13	5.55	5.13	4.72	O2	
4.60	4.93	4.56	4.30	O0	V2 eggplant
5.05	5.76	4.91	4.59	O1	

5.82	6.44	5.81	5.20	O2	
	5.40	4.91	4.53		average
0.0168		0.0291		0.0118	L.S.D

Average Varieties	S2	S1	S0	Algae Varieties
4.73	5.10	4.72	4.37	red V1
5.16	5.71	5.09	4.70	eggplant V2
	5.40	4.90	4.53	average
0.0097	0.0168			L.S.D

Average Organic Fertilizer	S2	S1	S0	Algae Organic Fertilizer
4.47	4.78	4.46	4.19	O0
4.88	5.42	4.78	4.44	O1
5.47	5.99	5.47	4.96	O2
		4.90	4.53	average
0.0118	0.0205			L.S.D

## Yield Characteristics and Components

### 1. Number of Capsules

The tables show significant differences between the two studied traits (red and eggplant). The eggplant variety (V2) outperformed the local red variety (V1) by achieving the highest average yield of 141.54 capsules per plant, while the red variety (V1) yielded the lowest average yield of 113.16 capsules per plant. The difference between the varieties is attributed to variations in their responses to environmental conditions and their genetic makeup. Furthermore, a variety that outperforms another in vegetative characteristics also outperforms it in marine characteristics, including the number of capsules. The overall increase in this trait was 20.05%.

The effect of adding seaweed extract varied depending on the concentration. Concentration (S2) outperformed the other concentrations, yielding the highest average of 163.31 plant capsules per 1000 plants, while concentration (S0) (the control treatment) yielded the lowest average of 124.18 plant capsules per 1000 plants. Since (S2) was superior in vegetative characteristics, it also outperformed (S0) in the number of capsules, a marine trait, resulting in a 23.96% increase.

The effect of adding organic fertilizers (cow manure) also varied depending on the concentration. O2 concentrations were the most effective, yielding the highest average of 164.44 plant capsules per 1000 plants, while concentration (S0), the control treatment, yielded the lowest average of 93.04 plant capsules per 1000 plants. In the dual interaction between the varieties and the organic fertilizer, the combination (O2×V2) was superior, yielding the highest average for the trait at 188.68 plant capsules per 1000 plants, while the combination (O0×V1) yielded the lowest average for the trait at 85.99 plant capsules per 1000 plants. A 54.42% increase in the trait was observed.

In the binary interaction between the varieties and the seaweed extract, the combination (S2×V2) was superior, yielding the highest average for the trait at 181.47 plant capsules per 1000 plants, while the combination (S0×V1) yielded the lowest average for the studied trait at 83.99 plant capsules per 1000 plants. Similarly, in the binary interaction between the organic fertilizer and the seaweed extract, the combination (S2×O2) was superior, yielding the highest average for the trait at 212.10 plant capsules per 1000 plants, while the combination (S0×O0) yielded the lowest average for the trait at 72.25 plant capsules per 1000 plants.

As for the three-way interaction between the experimental factors, the combination (S2×O2×V2) was distinguished by giving the highest averages for the trait, which amounted to 243.50 plant capsules-1, while the combination (S0×O0×V1) gave the lowest averages for the trait, which amounted to 66.26 plant capsules-1.

Average Varieties *Organic Fertilization	Seaweed Extract			Organic Fertilization	Varieties
	S2	S1	S0		

85.99	110.84	80.87	66.26	O0	V1 Red
113.29	143.93	111.22	84.73	O1	
140.20	180.70	138.93	100.73	O2	
100.09	127.33	94.69	78.25	O0	V2 Aubergine
135.85	173.57	136.44	97.53	O1	
188.68	243.50	182.94	139.61	O2	
	16.31	124.18	94.56		Average
1.421		2.461	1.005		L.S.D

Average Varieties	S2	S1	S0	Algae Varieties
113.16	145.15	110.34	83.99	Red V1
141.54	181.47	138.02	105.13	Aubergine V2
	163.31	124.18	94.56	Average
0.820	1.421			L.S.D

Average Organic Fertilizer	S2	S1	S0	Algae Organic Fertilizer
93.04	119.08	87.78	72.25	O0
124.57	158.75	123.83	91.13	O1
164.44	212.10	160.93	120.29	O2
	163.31	124.18	94.56	Average
1.005	1.740			L.S.D

### Anthocyanin%

The appendix and table show significant differences between the varieties. The eggplant variety (V2) gave an average for the trait of 0.284%, while the local red variety (V1) gave the lowest average for the trait at 0.230%, with an increase of 19.01%. The difference in the trait

between the two varieties can be attributed to their different genetic makeups, which are reflected in the physiological processes of each variety and thus affect the pigments they produce, including anthocyanin. The variety (V2), which excelled in all the studied traits, will certainly exhibit variation in anthocyanin content.

The addition of seaweed extract significantly impacted anthocyanin production. Concentration S2 yielded the highest average anthocyanin content at 0.317%, while treatment S0 (the control) produced the lowest average at 0.207%. This resulted in a 34.70% increase in anthocyanin production. This can be attributed to S2's superior performance across all studied traits, including its superior anthocyanin content.

However, the addition of organic fertilizer resulted in the O2 formulation outperforming the other treatments, yielding the highest anthocyanin content at 0.347%. Concentration O0 produced the lowest average anthocyanin content at 0.177%, resulting in a 48.99% increase in anthocyanin production. This can be explained by the fact that organic fertilizer contains macro- and micronutrients, as well as amino acids, which facilitate absorption by the gut. Regarding the interaction between certain varieties and organic fertilizer, the combination (O2×V2) was superior, yielding the highest average for the trait at 0.378%, while the combination (O0×V1) yielded the lowest average at 0.167%. A 55.82% increase in the trait was observed.

Regarding the interaction between certain varieties and seaweed extract, the combination (S2×V2) was superior, yielding the highest average at 0.352%, while the combination (S0×V1) yielded the lowest average for the trait at 0.189%. A 46.30% increase in the trait was observed.

Regarding the interaction between organic fertilization and seaweed, the (S2×O2) combination was superior, yielding the highest average for the trait at 0.421%, while the (S0×O0) combination yielded the lowest average at 0.149%. This resulted in a 64.60% increase in the trait.

As for the interaction between the three factors, the (S2×O2×V2) combination was superior, yielding the highest average for the anthocyanin trait at 0.455%, while the (S0×O0×V1) combination yielded the lowest average at 0.141%. This resulted in a 69.01% increase in the trait.

Average Varieties × Organic Fertilization	Seaweed Extract			Organic Fertilization	Varieties
	S2	S1	S0		
0.167	0.192	0.168	0.141	O0	V1 Red
0.216	0.266	0.189	0.177	O1	
0.315	0.387	0.310	0.250	O2	
0.188	0.223	0.184	0.157	O0	V2 Aubergine
0.289	0.381	0.279	0.207	O1	
0.378	0.455	0.371	0.310	O2	
	0.317	0.250	0.207		Average
					L.S.D

Average Varieties	S2	S1	S0	Algae Varieties
0.230	0.281	0.222	0.189	Red V1
0.284	0.352	0.278	0.224	V2 Aubergine
	0.317	0.250	0.207	Average
				L.S.D

Average Organic Fertilizer	S2	S1	S0	Algae Organic Fertilization
0.177	0.207	0.176	0.149	O0
0.249	0.323	0.234	0.192	O1
0.347	0.421	0.340	0.280	O2
	0.317	0.250	0.207	Average
				L.S.D

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