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Producing the organic Roselle crop and improving its growth, fruiting, and chemical constituents using a biostimulant compound.

Sudad K. Al-Taweel[1,*], Aws A. Mohammed[2], R.A.A. Al-Asadi[3]

[1,2]Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.

[3]Medical and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.

Corresponding author email: **S.K.T.:** sudad.altaweel@coagri.uobaghdad.edu.iq; **A.A.M.:** aws.a@coagri.uobaghdad.edu.iq; **R.A.A.A.:** rouaabd1983@gmail.com

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Abstract: Roselle (*Hibiscus sabdariffa* L.) is an annual shrub with a multipurpose crop with great economic potential to increase the income of farmers and processors in some countries worldwide from the export and local markets. Therefore, two successive seasons (2020 and 2021) were conducted to investigate how fertilization either organic manure or chemical fertilizer and foliar application of Appetizer (App) and their combination could affect the plant growth, yield, and chemical composition of Roselle plant. The treatments used App. solely at 3 rates (0, 4, 6 g L^{-1}), in interaction with the total dose of organic manure or chemical fertilizer, or with a half dose of both fertilizers. Also, values were tested for each fertilizer applied solely at a total dose and in interaction at a half dose. It was found that spraying Roselle plants with App positively increased all parameters studied over the control. However, such an increase was greatly enhanced when the App. was combined with fertilization. Whereas, the highest records of growth characters, the chemical constituents of roselle plant, ascorbic acid, and carbohydrate resulted from half doses of chemical and organic manure with 4 and 6 g L^{-1} of App. While the highest values of all yield characters were obtained from the combination of chemical fertilizer at total dose and App at 4 g L^{-1}, respectively, it was discovered, as well, that organic manure applied solely or combined with App gave high values that were so close and superior, in some cases, to those of chemical fertilizer. Hence, organically speaking, it is recommended that if there are fewer harmful and healthier products in agriculture, organic manure, either alone or combined with App, is still of high value for plant yield, especially when integrated with 4g L^{-1} App.

Keywords: Biostimulant, chemical Fertilizer, organic Fertilizer, roselle, seaweed extract, sustainable Agriculture.

Introduction

The medicinal plant *Hibiscus sabdariffa* L. is a member of the Malvaceae family. More people know it as "red sorrel" or "Roselle." There are more than 300 species and variations of hibiscus; the two most prevalent varieties are *H. sabdariffa* var. sabdariffa and *H. sabdariffa* var. altissima Wester. (Da-Costa-Rocha *et al.*, 2014). The annual shrub *Hibiscus sabdariffa* grows upright and has many branches. Reddish-brown stem with a taproot that penetrates deeply. Leaves with serrated edges that range in hue from a deep green to scarlet. Large, red to yellow flowers with a dark center on short peduncles are seen (Mady *et al*., 2009). Roselle is commonly consumed as food for medicinal and health purposes and is also a source of antioxidants, especially anthocyanins, which function as free radical scavengers and reduce lipid peroxidation; fascinating research on the antioxidant activity of Roselle extract and their consumption can reduce or protect from many diseases (Islam *et al*., 2016). Using roselle products, including fresh juice, hot and cold tea, jam, jelly, or capsules rich in anthocyanins, protects the human body from the detrimental effects of free radicals through antioxidant activity. The economic part of the plant is the fleshy calyx surrounding the fruit (capsules); it is well-known for its juicy, edible calyces and leaves, which can be used to manufacture ice cream, salads, tea, juices, jams, and jellies, among many other items (Yirzagla *et al*., 2023). Because of their high ascorbic acid and anthocyanin content, fresh roselle calyces are gathered in various places to make pro-health drinks. According to studies by Malacrida *et al.* (2022) and Sehim *et al.* (2023) Roselle calyxes have a progressive function in the development of human breast cancer cells, in addition, it finds application in the pharmaceutical sector. Infusions made from the calyces or leaves are used in traditional medicine in India, Africa, the Middle East, and Mexico to treat fever, hypertension, hypercholesterolemia, liver diseases, and gastrointestinal disorders. According to nutritionists, Roselle calyces are rich in Potassium, calcium, magnesium, sodium, riboflavin, niacin, and iron. Table 1 displays the nutritional makeup of 100 g of fresh Roselle calyces and leaves. Roselle plant can be grown as a supplementary crop on the farm to boost income; it is drought tolerant and requires minimal attention and inputs once established.

Table (1): Nutritional composition of 100 g fresh Roselle calyces and leaves (Islam *et al*., 2016).

Constituents	Fresh Calyx	Fresh leaves
Moisture	9.20 g	85.60 g
Protein	1.15 g	3.30 g
Fiber	12.00 g	10.00%
Fat	2.61 g	0.30 g
Fiber	12.00	10.00%
Energy	44 kcal	43 kcal
Ash	6.90 g	1.00 g
Calcium	12.63 mg	213.00 mg
Phosphors	273.20 mg	93.00 mg
Iron	8.98 mg	4.80 mg
Carotin	0.03 mg	4135 μg
Thiamin	0.12 mg	0.2 mg
Riboflavin	0.28 mg	0.45 mg
Niacin	3.77 mg	1.2 mg
Carbohydrate	6.70 mg	154 mg
Ascorbic acid	10.00 mg	9.20 g

These attributes may be ascribed to its nutritional content. Roselle possesses a high concentration of organic acids, amino acids, minerals, carotene, vitamin C, and total sugars in its calyx, leaves, and seeds, with variations dependent on variety and geographical location in addition to flavonoids, triterpenoids, anthocyanins, steroids, and alkaloids (Mady *et al*., 2009). Chemical fertilizers are well recognized to be two-edged swords. They are crucial elements of contemporary agriculture (Adesemoye *et al*., 2009) to achieve food security (Kiran *et al*., 2016). The results exhibited by (Ali *et al.,* 2021) showed significant differences in the applying N fertilizer rate of nitrogen and increased the vegetative growth and yield. However, they also exhibit several adverse effects. Over time, regular, uneven application of chemical fertilizers causes the soil to become less base-saturated and more acidic (De-Ridder & Van-Keulen, 1990). Chemical fertilizers adversely influence the environment and public health through leaching, soil degradation, groundwater contamination, and accelerated nutrient release (Mohammed *et al*., 2020).

A test for the nutritional content of the soil and manure could assist in avoiding this issue by identifying the proper requirements and quantity of manure required. In order to restore the ecological balance that was upset by improper practices of an organic, sustainable agroecosystem, there has been a trend toward using fertilizers derived from natural sources, as these fertilizers could provide renewable and organic sources of nutrients for crops (Roe, 1998).

The main reasons for using organic farming systems include food safety, reducing adverse environmental effects, enhancing the nutritional attributes of organic products, and producing more palatable and healthier food items (Youssef *et al*., 2014). Furthermore, a crucial objective is to improve soil structure and stability, thereby increasing crop yield and quality (Chang *et al*., 2010). Excessive manure application may postpone ripening and heighten susceptibility to disease, frost, and wind damage. For a long time, soil fertility preserved by using cattle manure, an animal waste product (Castrillón *et al*., 2002). It comprises manure and excrement, bedding, residual feed, and water. Many minerals essential for plant growth found in cattle manure.

It can replace or lessen the demand for commercial nutrients in crop cultivation due to its high level of organic matter. Nevertheless, cow dung has a modest healthy content, so large application rates are necessary to apply the same amount of nutrients. Both organic and inorganic nutrients can be found in animal dung. The organic portion of solid manure unavailable to plants contains a high percentage of nutrients. They must first undergo mineralization to convert nutrients from their organic to inorganic forms. Ten to twenty percent of the immediately available Nitrogen is found in the inorganic portion of solid cattle dung. Compared to commercial phosphorus fertilizers, the availability of Phosphorus in cattle manure is around 50%, and other elements in the manure, such as Nitrogen, affect how the Phosphorus reacts (SSCA, 2000).

Presently, there is a global movement to use nano fertilizers and natural Biostimulants to decrease synthetic fertilizers' usage because of their negative effects on the environment, human health, and the economy (Al-Taweel & Mohammed, 2023; Al-Rawi *et al*., 2024). One such natural alternative is using seaweed extracts, which are inexpensive, non-toxic, and do not pollute the environment (Priyanka *et al*., 2020). Additionally, these substances promote plant growth at a low concentration and support any crop's most critical physiological processes. An appetizer is an example of a seaweed extract used to achieve this goal: a biostimulant sprayed on the shoots and used as a seed soak before seeding. The natural biostimulator Appetizer is derived from the seaweed *Ascophyllum nodosum* L., constituting 93% of the commercial product. The chemical composition of the seaweed as shown in Table 2. These algae possess numerous biologically active phenolic compounds and certain plant hormones, including gibberellins, cytokinins, auxins, and other components. The minerals zinc and manganese are present in addition to a high content of phenolic compounds, including phlorotannin and alginic acid, around 28% (Saudi & Al-Rawi, 2023). Seaweed extract enhances plant growth, productivity, and quality through the presence of many plant growth hormones and regulators, including gibberellins, cytokinins, auxins, abscisic acid, betaines, brassinosteroid, Jasmonate, polyamines, salicylates, and signal peptides (Almafrajee & Elrubaee, 2022). Seaweed contains various natural compounds and bioactive substances, including terpenoids, carotenoids, polyphenols, peptides, sulfated polysaccharides, and fibers (Bruno *et al*., 2023). it is important for many physiological processes in crops. The results obtained by Almafrajee and Elrubaee (2022) showed that applying the organic Appetizer enhanced the growth, quantity, quality, and active components of maize silk in synthetic maize cultivars. This field study looked into the viability of producing organic Rosselle and how an appetizer (App) foliar spray could enhance plant growth, fruit productivity, medicinal properties, and chemical makeup.

Materials & Methods

A field experiment was conducted during two seasons (2020 & 2021). In every season, seeds of Roselle (*Hibiscus sabdariffa*, L. cv. Egyptian Balady variety red light) plants were kindly obtained by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Egypt. Seeds were sown in Sandy clay soils at the first week of May. The experiment was laid out as a split-plot design based on completely randomized blocks with three replicates. The plot area was (2.80 × 3.60) = 10.08 m^2 and included six rows; each row was 60 cm apart and 2.80 m in length, every row had eight hills (40 cm apart), and three weeks later, the plants were thinned, leaving only one seedling per hill. Seeds. The thinning was cut three weeks later, leaving each hill with just one seedling. The soil's physical and chemical analyses were conducted following the methodologies that Jackson (1973) and Black *et al*., (1982) established, respectively. The soil analysis findings are provided in Table 2. The primary experimental design involved three fertilizing treatments: the first treatment consisted of 100% organic fertilizer and 0% chemical fertilizer, explicitly using 60 m^3 of cattle manure per hectare without any chemical fertilizer.

Table (2): Experimental soil characteristics:

Trait	2020	2021
Sand %	12	10
Silt %	22	25
Clay %	68	61
Texture	Clay	Clay
N	17.5	18.7
P	70.3	77.9
K	93.9	99.9
Fe	3.7	3.8
Zn	0.90	0.99
Mg	0.40	0.88
EC dSm^{-1}	1.88	1.92
Organic matter %	1.11	1.16

The second treatment consisted of a 50% organic fertilizer and a 50% chemical fertilizer. The chemical fertilizer included 50 kg per hectare of Urea (containing 46.5% nitrogen), 60 kg per hectare of di-ammonium phosphate (containing 46% P_2O_5), and 112.5 kg per hectare of potassium sulfate (containing 48% K_2O). Additionally, 30 m^3 per hectare of bovine manure was applied. The third treatment consisted of a complete application of chemical fertilizers, including 100 kg $hectare^{-1}$ Urea (46% N), 125 kg ha^{-1}, di-ammonium phosphate (46% P2O5) and 225 kg ha^{-1} potassium sulfate (48% K_2O), without the addition of organic manure. The Nitrogen and potassium fertilizers were divided into three equal quantities and applied as a side dressing at three different periods during the last week of June, July, and August in both seasons at same the same experiment site and experiments units. Before sowing the seeds, a specific quantity of P-fertilizer and organic manure (OM) were applied to the soil during the process of soil preparation. The chemical characteristics of the analyzed cattle manure (CM) are displayed in Table 3. The subplot was devoted to three Biostimulants (Seaweed Table 3) App sprays, 0, 4, and 6 g L^{-1}.

Table (3): The physical and chemical properties of cattle manure used

Fertilizers characteristics	Value
Weight (kg /m^3)	427
Moisture (%)	8.11
Ash (%)	30.48
C/N ratio	17:1
Organic matter (%)	8.15
Organic Carbon (%)	27.11
Total Nitrogen (%)	3.28
Total Phosphorus (%)	0.95
Total Potassium (%)	0.90
Ammonium (ppm)	3.83
Nitrate (ppm)	0.94
Fe (ppm)	222
Zn (ppm)	90.17
Mn (ppm)	33.11
Cu (ppm)	32.77

The natural biostimulator Appetizer is derived from the seaweed *Ascophyllum nodosum* L., constituting 93% of the commercial product the chemical composition of the seaweed is shown in Table 4. The plants were sprayed three times during the vegetative growth, stem elongation, and flowering; the sprays were applied in the morning using a hand pressure sprayer (Atteya *et al*., 2018). The control plants were treated with distilled water. The spraying solution volume was adjusted to ensure thorough coverage of the plant leaves without any excess that would cause dripping. All agricultural techniques, including cultivation, irrigation, and pest control, were carried out.

Ten plants were selected from each treatment to determine the studied characters as follows:

Growth parameters

Plant height (cm) and fresh and dry weight of the plant (g plant^{-1}) were taken at the beginning of the flowering stage during September of both seasons (2020 and 2021).

Yield Characters

Fruit fresh weight (g plant^{-1})**,** Number of fruits plant^{-1}, sepals fresh and dry weight (g plant^{-1}) were recorded at harvesting time. (at the last week of October 2020-21) seasons.

Chemical constituents

The leaves' total carbohydrates (mg g^{-1} DW) were quantified at the beginning of the flowering stage, as per Herbert *et al*. (1971). The chlorophyll meter (SPAD-504, Konica Minolta Sensing, Inc., Japan) was used to evaluate the leaf chlorophyll index (SPAD). The ascorbic acid content of fresh calyx of the plant was quantified (mg g^{-1} fresh weight) utilizing the 2,6-dichlorophenol indophenol method as outlined by A.O.A.C. (2023).

Table (4): Chemical composition (%) of seaweeds *Ascophyllum nodosum* (Priyanka *et.al*, 2020)

Parameter(s)	*Ascophyllum nodosum*
Type	Brown
Water	70-85
Ash	15-25
Alginic acid	15-30
Laminaren	0-10
Mannitol	5-10
Fucoidan	4-10
Carbohydrates	10
Protein	5-10
Fat	2-7
Tannins	2-10
Potassium	2-3
Sodium	3-4
Magnesium	0.5-0.9
Iodine	0.01-0.1

Statistical analysis

The Genstat program statistically analyzed the experimental data, and the least significant difference (LSD) test at a 0.05 probability level was employed to compare means (Steel & Torrie, 1980).

Results & Discussion

Growth parameters

Organic and chemical fertilizers

According to Table 5, there were no appreciable variations in plant height throughout the two study seasons. The highest significant plant height, measuring 181.66 cm, obtained by applying organic manure at a full dosage in the first season and mixing half doses of chemical fertilizers and organic manure (195.64 cm) in the second season. Compared to the outcomes of the other treatments, combining both fertilizers at half doses resulted in the greatest fresh weight of 523.45 and 909.02 g plant $^{-1}$ in the first and second seasons, respectively. As for dry weight per plant, the highest significant dry weight per plant, measuring 135.94 g plant^{-1}, obtained by applying mixing half doses of chemical fertilizers and organic manure in the first season and organic manure at a full dosage (215.73 cm) in the second season.

Appetizer

Biostimulant appetizers significantly increased plant height (181.45 and 195.09) when sprayed at a 6 and 4 g L^{-1} in the first and second seasons, respectively. As for fresh and dry weight per plant, the spraying of App at a 4 g L^{-1} significantly excelled in the first season (587.00 and 135.38 g plant^{-1}), whereas the spraying it at a 6 g L^{-1} significantly excelled in the second season (770.18 and 204.85 g plant^{-1}) for two traits respectively.

Interaction between Fertilizers and Appetizer

It could be inferred that spraying Roselle plants with a Biostimulant Appetizer and fertilization increased the growth parameters studied. As for plant height and plant fresh weight, the spraying of App at a 6 g L-1 with the addition of organic manure at a full dosage gave significant values in the first season (189.33 and 599.00 g plant^{-1}), while the spraying of App at a 4 g L-1 with the addition of mixing half doses of chemical fertilizers and organic manure gave significantly values plants in the second season (214.60 cm and 990.70 g plant^{-1}). Regarding plant dry weight, the spraying of App at a 6 g L-1 with the addition of mixing half doses of chemical fertilizers and organic manure gave significant values in the first season (154.90 g plant^{-1}). In comparison, the spraying of App at a 6 g L-1 with the addition of organic manure at a full dosage gave significantly values in the second season (290.89 g plant^{-1}).

Yield Characters

Fertilization with organic and chemical fertilizers

The plants amended with cattle manure at complete dose solely gave the highest sepals fresh weight (192.22 and 283.56 g plant^{-1}), sepals dry weight (34.53 and 42.94 g plant^{-1}) fruit number (60.40 and 88.46 fruits plant^{-1}) and fruits fresh weight (363.43 and 570.50 g plant^{-1}) in both seasons respectively.

Appetizer

According to the data presented in Table 6, the spraying of App at a 4 g L^{-1} was significantly superior by giving the highest means of sepal's fresh weight (186.11 and 283.12 g plant^{-1}), sepals dry weight (32.94 and 42.00 g plant^{-1}), fruit number (63.22 and 92.11 fruits plant^{-1}), and fruits fresh weight (362.06 and 559.30 g plant^{-1}) in both seasons, respectively.

Interaction between fertilizers and Appetizer

The interaction between fertilization and appetizer concentrations had a significant effect on the yield characters, as the spraying of App at a 4 g L^{-1} with the addition of organic manure at a full dosage gave the highest values of sepals fresh weight (219.00 and 315.00 g plant^{-1}), sepals dry weight (37.93 and 45.49 g plant^{-1}) fruit number (72.33 and 99.67 fruits plant^{-1}) and fruits fresh weight (396.30 and 618.40 g plant^{-1}) in both seasons respectively.

Table.5. Effects of fertilization and Appetizer treatments and their interaction on some Roselle growth traits

Plant height (cm)								
	1st Season				2nd season			
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	167.00	165.00	171.67	167.89	181.33	179.00	196.67	185.67
4	188.67	185.00	170.67	181.45	191.00	214.60	179.67	195.09
6	189.33	166.67	175.33	177.11	185.67	193.33	191.67	190.22
Mean	181.66	172.22	172.56		186.00	195.64	189.34	
LSD.05	Ferti = 7.18	App = 5.47	A × F = 12.31		Ferti = 8.33	App = 6.51	A × F = 15.28	
Plant FW (g plant^{-1})								
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	260.69	550.68	333.67	381.68	602.36	802.69	347.10	584.05
4	366.33	435.33	587.00	462.88	638.67	990.70	535.53	721.63
6	599.00	584.33	510.69	564.67	926.65	933.68	450.23	770.18
Mean	408.67	523.45	477.12		722.56	909.02	444.28	
LSD .05	Ferti = 29.96	App = 23.42	A × F = 49.74		Ferti = 33.81	App = 29.72	A × F = 64.47	
Plant DW (g plant^{-1})								
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	79.25	142.74	117.33	117.53	210.86	203.36	100.99	171.74
4	90.35	110.17	135.38	105.95	145.45	218.19	198.07	187.24
6	80.56	154.90	130.16	123.61	290.89	214.69	108.98	204.85
Mean	83.39	135.94	127.77		215.73	212.08	136.01	
LSD.05	Ferti = 6.06	App = 4.98	A × F = 8.38		Ferti = 11.52	App = 8.08	A × F = 20.17	

Table (6): Effects of fertilization and Appetizer and their interaction on Roselle yield characters

Sepals FW (g plant^{-1})								
	1st Season				2nd season			
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	159.33	157.00	99.67	138.66	275.68	226.67	189.00	230.45
4	219.00	163.00	176.33	186.11	315.00	235.00	299.36	283.12
6	198.33	167.00	179.00	178.22	260.00	251.00	281.33	270.12
Mean	192.22	162.44	158.11		283.56	237.55	256.56	
LSD.05	Ferti = 11.22	App= 7.46	A × F = 17.61		Ferti = 17.63	App = 10.56	A × F = 24.72	
Sepals DW (g plant^{-1})								
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	27.76	24.88	31.47	28.04	40.80	38.88	32.30	37.32
4	37.93	26.45	34.44	32.94	45.49	36.85	43.67	42.00
6	37.90	25.60	31.80	31.76	42.55	41.00	40.57	40.34
Mean	34.53	25.64	32.57		42.94	38.91	38.80	
LSD .05	Ferti = 1.97	App = 1.32	A × F = 2.41		Ferti = 2.06	App = 1.73	A × F = 3.18	
Fruits number (fruit plant^{-1})								
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	50.55	46.67	42.67	46.63	82.38	76.00	66.83	75.07
4	72.33	51.00	66.33	63.22	99,67	82.00	94.67	92.11
6	58.33	53.00	58.37	55.47	83.33	81.33	78.00	77.00
Mean	60.40	50.22	55.79		88.46	79.78	79.83	
LSD.05	Ferti = 3.02	App = 2.16	A × F = 4.43		Ferti = 4.21	App = 3.52	A × F = 6.96	
Fruits FW (g plant^{-1})								
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	320.40	318.70	321.26	320.12	522.70	289.30	444.0	418.66
4	396.30	296.60	393.30	362.06	618.40	464.70	594.80	559.30
6	374.00	291.50	336.00	333.83	570.40	499.80	520.70	530.30
Mean	363.43	302.30	350.18		570.50	417.93	519.83	
LSD.05	Ferti = 17.2	App = 13.9	A × F = 26.7		Ferti = 25.8	App = 19.5	A × F = 43.1	

Chemical composition

Fertilization with organic and chemical fertilizers

The applying of mixing half doses of chemical fertilizers and organic manure significantly excelled in the chemical composition of Roselle by achieving the highest chlorophyll index (42.81 and 45.90 SPAD), Ascorbic acid (27.95 and 26.98 mg g^{-1} FW) and Total Carbohydrates (189.96 and 203.18 mg g^{-1} DW) in both seasons respectively (Table 7).

Appetizer

The results of Table 7 show that the spraying of App at a 6 g L^{-1} significantly excelled in the chemical composition of Roselle by giving the highest means of chlorophyll index (43.48 SPAD) in the first season only, ascorbic acid (27.70 and 26.75 mg g^{-1} FW) and total carbohydrates (187.77 and 196.14 mg g^{-1} DW) in both seasons respectively. However, the spraying of App at a 4 g L^{-1} gave a highest mean of chlorophyll index (46.07 SPAD) in the second season.

Interaction between fertilizers and Appetizer

The interaction between studied factors had a significant effect on the chemical composition, as the spraying of App at a 6 g L^{-1} with the application of mixing half doses of chemical fertilizers and organic manure gave the highest values of chlorophyll index (43.97 SPAD) in the first season only, ascorbic acid (30.90 and 29.44 mg g^{-1} FW) and total carbohydrates (212.33 and 220.93 mg g^{-1} DW) in both seasons respectively. However, the spraying of App at a 4 g L^{-1} with mixing half doses of chemical fertilizers and organic manure gave the highest chlorophyll index (48.46 SPAD) in the second season.

Table (7): Effects of fertilization and App and their interaction on Roselle chemical composition

Chlorophyll index (SPAD)								
	1st Season				2nd season			
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	40.89	43.05	39.31	41.08	39.26	43.43	39.11	40.60
4	42.54	41.42	44.03	42.66	42.92	48.46	46.84	46.07
6	42.36	43.97	44.11	43.48	45.90	45.83	41.66	44.46
Mean	41.93	42.81	42.48		42.69	45.90	42.53	
LSD.05	Ferti = N.S.	App = N.S.	A × F = 3.54		Ferti = 2.58	App = 1.82	A × F = 4.03	
Ascorbic acid (mg g^{-1} FW)								
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	22.60	25.55	24.78	24.31	21.18	25.14	25.57	23.96
4	23.05	27.41	25.12	25.19	21.95	26.36	25.79	24.70
6	24.35	30.90	27.85	27.70	25.41	29.44	25.41	26.75
Mean	23.33	27.95	25.92		22.85	26.98	25.59	
LSD .05	Ferti = 1.82	App = 1.34	A × F = 2.29		Ferti = 1.68	App = 1.43	A × F = 2.84	
Total Carbohydrates (mg g^{-1} DW)								
App (g L^{-1})	Ferti			Mean	Ferti			Mean
	Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.		Org.100% + 0% chem	Org.50% + 50% chem.	Org. 0% + 100% chem.	
0	147.88	158.86	184.97	163.90	158.57	181.39	191.98	177.31
4	160.13	198.80	185.70	181.54	165.17	207.23	184.80	185.73
6	166.00	212.33	190.39	187.77	178.37	220.93	189.13	196.14
Mean	158.00	189.96	187.02		167.37	203.18	188.63	
LSD.05	Ferti = 14.53	App = 11.98	A × F = 20.17		Ferti = 11.16	App = 9.44	A × F = 17.83	

Discussion

Cattle manure is an organic compound rich in Nitrogen, Potassium, calcium, and Phosphorus. Moreover, varying proportions of various substances are present in it, primarily consisting of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) (Gendy *et al*., 2012). So, applying manure to agricultural soils is a tried-and-true method of increasing soil organic matter, a vital component of soil health, and restocking essential plant nutrients, such as nitrogen, for healthy plant growth (Moncayo & Dreyer, 2018; Abraham *et al*., 2024). Bogaard *et al*. (2013) reported that adding growth-promoting agents, like hormones and enzymes, can enhance the way crops respond to fertilizers and help restore soil productivity in areas that have been degraded. It improves the soil's chemical, physical, and biological properties (Benke *et al*., 2008) by boosting macrospores and hydraulic conductivity (ChauDhary & Narwal, 2005), lowering microspores, and enhancing soil stability. Thus, this could account for the better performance and superiority of organic manure over chemical fertilizer in terms of growth traits and yield attributes (such as the weight of fresh fruit, the Number of fruits, and the dry weight of sepals). However, there was no statistically significant difference in the values between the two fertilizers for the remaining evaluated parameters. These results align with the conclusions drawn on Roselle by Attia & Khater (2015), Gendy *et al*., (2012), and Yusuf & Adzemi (2019). It was found that using a higher rate of organic fertilizer increased the stem diameter and stem height, leaves number and leaves area, as well as the biomass and calyx number of Rosselle (Yusuf & Adzemi, 2019). According to the findings, most values showed a noteworthy rise in the second season as opposed to the first. The delayed manifestation of organic manure's effects is because nutrients are released gradually and consistently over an extended period. By promoting the activity of soil microbial biomass, this mechanism improves soil fertility (Ayuso *et al*., 1996).

According to earlier studies, using organic and inorganic fertilizers can increase crop output, preserve soil fertility and health, and produce the greatest yield and return. Also, this combination can aid in addressing certain dietary deficiencies that result in ill health and decreased productivity. Adeniyan & Ojeniyi (2005) suggest using organic fertilizer and chemical fertilizer to close the gap between supply and demand for fertilizer while considering the environment and economy. According to Yeshiwas *et al*., (2018), adding organic manure in addition to inorganic fertilizers can aid in restoring the soil's synthetic N, P, and K levels, which are rapidly depleted. Chemical fertilization has a problem; this is due to volatilization and leaching of N in chemical nitrogen fertilizers such as Urea or due to fixation in the soil as in the element's phosphorus, potassium, and some minor elements, depending on the type of soil and its physical and chemical characteristics. As a result, the combination of the two fertilizer types half doses from organic and chemical fertilizers with App. at the highest rate (6 g L^{-1}) was found to be highly advantageous for stimulating growth, affecting the dry weight percentage, significantly increased percentage of Ascorbic acid the non-enzymatic oxidant scavenger with increasing ascorbate peroxidase and enhancing specific yield characteristics (Al Taweel *et al*., 2022). In their study on Roselle and Carrot (Youssef *et al*., 2014), (Kiran et al., 2016), claimed that giving plants a full dose of manures together with NPK delivered the essential macronutrients, increasing leaf yield, further corroborating this idea. Furthermore, comparable findings were also reported by (Asmaa *et al.*, 2014). Compared to organic manure, chemical fertilizer showed a slight improvement in most traits but not all of them. Yusuf & Adzemi (2019) indicated that plant growth and yield were generally significantly increased in Roselle treated with organic fertilizer, increased stem diameter and height, Number and area of leaves, biomass, and Number of calyxes .

Adding specific yield metrics, such as the weight of the seeds and the fresh weight of the sepals, and thoroughly examining all chemical constituents improved the information's clarity. Essential plant nutrients are readily available to plants due to the presence of NPK, which is highly soluble in soil solutions (Yeshiwas *et al*., 2018). This aids in the plant's ability to make up for any inadequacies (Soro *et al*., 2015). Many amino acids, enzymes, and molecules that transport energy, such ATP, ADP, and chlorophyll, all contain Nitrogen. Therefore, as indicated by (Bidwell, 1974), a large nitrogen shortage will entirely interrupt the processes of development and reproduction .

Furthermore, it has been demonstrated that giving plants enough Nitrogen at the correct times increases the quantity and size of cells, which increases the overall production of vegetative growth. Cell division and the development of meristematic tissues depend on Phosphorus (Fathi, 2022). It is essential for the creation of energy-dense phosphate linkages and the conversion of carbohydrates through a variety of phosphorylation pathways (Lambers *et al*., 2008). In the cell, Phosphorus joins forces with Nitrogen, oxygen, hydrogen, carbon, and other elements to form complex organic molecules. Phosphorus molecules are essential for the metabolism of lipids, amino acids, and cellular oxidation, in addition to photosynthesis (Ibrahim *et al*., 2020). A deficiency in Phosphorus hinders metabolic processes, such as the transformation of carbohydrates into starch and cellulose. Because it functions as an osmotic agent and might work in concert with IAA, Potassium is essential for encouraging growth and elongation (Hasanuzzaman *et al*, 2018). Moreover, it facilitates the transfer of carbohydrates from leaves to storage tissues and the assimilation of CO2. The results obtained from this investigation are consistent with those published by (Youssef *et al*., 2014) with Roselle ,

Moreover, an interaction existed between the two parameters; the studies of Akanbi *et al*., (2009) and Ibrahim *et al*., (2020) indicated that the application of chemical fertilizer involved 0, 40, 80 kg N fed-1 and 40 N + 40 kg P2O5 fed-1. In contrast, fermented organic fertilizers were 2-ton chicken manure fed-1 and 3-ton cattle manure fed-1, significantly affecting the number of per plant only. The findings align with Yirzagla et al., (2023), who recommended an application rate of 2.5 t ha-1 of cow dung and 60 kg ha-1 of nitrogen for optimal Roselle production.

Appetizer is one of the seaweed extracts employed for Biostimulator when employed in minimal quantities, it is considered a substance that enhances plant growth due to its content of various hormones, including auxins, cytokinins, and GA3 (Saudi & Al-Rawi, 2023). Furthermore, it encompasses a range of macronutrients, vital amino acids and vitamins. These components are essential for fostering growth and facilitating vital physiological functions in the crop. Consequently, it fosters the advancement of the vegetative system and the plant's root system, improves the roots' efficiency in water and mineral absorption, and delays the aging process of the roots (Kasim *et al*., 2015).

Also, the increase in plant yield when treated with appetizer can be attributed to its content of nutrients, as these nutrients provide essential elements and work in coordination with the plant's ability to absorb nutrients from the soil, ensuring the entire plant is equipped with all the necessary nutrients (Al-Taweel & Mohammed, 2023). In addition, they have a critical function in controlling cell components and enhancing the plant's resistance to environmental stresses like salt stress and drought stress (Kasim *et al*., 2015). Moreover, they also cause an increase in the chlorophyll content of leaves and the rates of carbon metabolism. It also functions as an antioxidant by boosting the activity of certain enzymes. It is important for regulating cells and enhancing plant resistance to environmental stressors, including salinity and drought.

On the other hand, the results show that most values greatly increased during the second season compared to the first. This may be because the effect of organic manure takes a long period of time to appear, as the organic manure releases nutrients rather slowly and steadily over a longer period. This, in turn, improves the soil fertility status by activating the soil microbial biomass (Ayuso *et al*., 1996).

Conclusion

It is noticed that the effect of the App as a sole treatment greatly enhanced all attributes studied, especially yield attributes, when the app was combined with fertilization. However, when combined with half doses of both fertilizers, it gave high values for most growth characters of Roselle sepals and some chemical constituents such as ascorbic acid. This emphasizes that Appetizer greatly influences the characters studied and could be used effectively in organic agriculture, which puts chemical fertilizers aside. However, a high concentration of it reduced the values of some attributes, especially those of plant yield. Therefore, a moderate rate of App (4 g L^{-1}) is recommended for a higher yield, but this was different for the chemical composition, which responded greatly to the higher concentration (6 g L^{-1}).

Moreover, organic manure showed positive effusive effects, especially in total dosages for all yield attributes and half dosages for chemical composition. However, such an increase could be favorable over that increase in unhealthy and unsafe chemically amended products. Organic manure, solely or with the assistance of the App, can increase the yield of Roselle with the advantage of having a high-quality and medicinal value product without endangering either the soil or the living beings to any harm the chemical fertilizers. Partial substitution of chemical fertilization of 50% of Roselle plant by organic fertilization and sprayed three times of App (4 g L-1) during vegetative growth stages, stem elongation. Flowering is recommended for higher yield and quality of roselle production and for maintaining human health for sustainable agriculture.

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Contributions of authors

S.K. Al-Al-Taweel: Suggestion the proposal of the article, adviser of fields experiment, wrote and revised the manuscript.

A.A. Mohammed: Statical analysis, wrote and revised the manuscript.

R.A.A. Al-ASadi: Chemical analysis, revised the manuscript.

ORCID

S. K. Al-Taweel: https://orcid.org/0000-0003-2295-0990

A.A. Mohammed: https://orcid.org/0009-0006-0898-6355

R.A.A. Al-Asadi: https://orcid.org/0000-0002-1200-9788

Conflicts of interest

The authors declare that they have no conflict of interests.

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إنتاج محصول الكجرات العضوي وتحسين النمو والإثمار والمكونات الكيميائية بالرش بالمحفز الحيوي

سداد كاظم الطويل[1] و اوس علي محمد [2] و رؤئ عبد الحسين الاسدي[3]

[2،1] قسم المحاصيل الحقلية، كلية علوم الهندسة الزراعية، جامعة بغداد

[3]وحدة بحوث النباتات الطبية والعطرية ، كلية علوم الهندسة الزراعية، جامعة بغداد

المستخلص: الكجرات (*Hibiscus sabdariffa* L.) شجيرة حولية متعددة الاستعمالات ذات مردود اقتصادي كبير في زيادة دخل المزارعين والمصنعين في بعض الدول حول العالم سواء في الأسواق المحلية او للتصدير. أُجريت دراسة لموسمان متتاليان (2020 و2021) لمعرفة دور التسميد، سواءً بالسماد العضوي أو الكيميائي، والرش الورقي بمُستخلص المحفز الحيوي Appetizer (App)، في نمو نبات الكجرات وفي إنتاجيته وتركيبه الكيميائي. رش مُستخلص Appetizer بثلاثة مستويات (0، 4، 6 غم لتر$^{-1}$)، بالتداخل مع الجرعة الكلية من السماد العضوي أو الكيميائي، أو مع نصف الجرعة من كلا السمادين. كما تم اختبار اضافة كلا السمادين لوحده بكامل الجرعة وبنصف جرعة. أظهرت النتائج أن رش نباتات الكجرات بامُستخلص الحيوي Appأدى إلى زيادة ملحوظة في جميع الصفات المدروسة مقارنةً بالنباتات غير المعاملة به (المقارنة)، كما ولوحظ زيادة في هذه الصفات وبشكل عام عند رش App بالتداخل مع التسميد. أن أعلى معدلات في نمو النبات ومحتواه من حامض الأسكوربيك والكربوهيدرات وجدت عند أضافة نصف الجرعة من السماد الكيميائي والعضوي وعند الرش بتركيز 4 و6 غم لتر$^{-1}$ من المحفز الحيوي. تم الحصول على أعلى قيم لجميع صفات الحاصل من اضافة السماد الكيميائي بكامل الجرعة والرش بالمحفز الحيوي بتركيز 4 غم لتر$^{-1}$ على التوالي. أظهرت النتائج أن السماد العضوي المضاف إليه السماد العضوي فقط أو مع الرش بالمستخلص الحيوي App قد أعطى قيمًا عالية قريبة جدًا ومتفوقة في بعض الحالات على أضافة الاسمدة الكيميائية منفردة. لذا، يُوصى بأنتاج الكجرات بالتسميد العضوي سواء لوحده او مع الرش بالمحفز الحيوي Appبمعدل 4 غم لتر$^{-1}$ وانتاج محصول صحي دون اي اضرار جانبية على الانسان.

<u>الكلمات المفتاحية:</u> الكجرات، التسميد العضوي، التسميد الكيميائي، المُحفز الحيوي، مستخلص الاعشاب البحرية، زراعة مستدامة.