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Using of blue green algae extract and salicylic acid to mitigate heat stress on roselle (*Hibiscus sabdariffa* L.) plant under Siwa Oasis conditions

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Abstract

Increasing temperature is a global issue due to the existing climate change problem that reduces agricultural productivity and increases prices. It badly affects the yield, and active constituents of medicinal and aromatic plants, especially in newly reclaimed lands in desert areas. The *Hibiscus sabdariffa* L. is a remarkable crop known for its calyces to make a refreshing drink. It is in other food industries and has many healing effects. A split-plot field experiment was conducted on *Hibiscus sabdariffa* L. at Siwa Oasis, Egypt, during the 2018 and 2019 seasons for studying to reduce the harmful effect of heat stress on plants and yield improvement. Spraying with three salicylic acid concentrations was put to main plots as 0, 500 and 1000 mg/l and the number of sprays with *Spirulina platensis* algae extract was allotted to subplots and used as 0, 1 and 2 times in the season. Some quantity and quality parameters were under investigation. The significant highest increases in dry weight per plant, number of fruits per plant, dry yield of sepals per hectare and sepal's anthocyanin content were from the combination among spraying with salicylic acid at the concentration of 500 mg/l and spraying with blue green algae extract twice through the season. This treatment was helpful to decrease the high temperature injury on growth through the summer months.

Keywords

High temperature, *Hibiscus sabdariffa*, salicylic acid, *Spirulina platensis* extract

Introduction

Hibiscus sabdariffa L. is an essential plant that belongs to the Malvaceae family and is successfully grown in tropical and subtropical climates. It is a popular drink in Egypt. Sepals are a commercially valuable part of the plant, and their color plays a role in determining the quality of *H. sabdariffa*. Sepals have some medicinal uses and food preparation in sauces, jams, juices, jellies, syrups, flavoring and coloring agents for food and drinks. The sepals are rich in secondary metabolites such as organic acids and vitamin C. Also, they contain two anthocyanins, namely delphinidin-3-sambubioside (hibiscin) and cyanidin-3-sambubioside (gossypicyanin) (1, 2). Secondary metabolites of medicinal and aromatic plants have great pharmaceutical importance. *H. sabdariffa* L. juice has a strong antioxidant properties and antiproliferative activity on some cancer cell lines, which correlate mainly to its anthocyanin content (3-6).

Heat stress is one of the majority of abiotic plant stresses that will increase due to global climate change. High temperatures in tropical climates and temperate regions are often the most limiting factors affecting plant growth and crop yield. They can cause considerable damage, including scorching leaves and twigs, sunburn on leaves, branches, stems, leaf senescence, abscission and reduced yield by decreasing net photosynthesis. High temperature negatively affects plant growth and survival and causes a decrease in crop yield. Photosynthesis is particularly sensitive to heat stress. The recent results provide important new insights into the mechanisms by which moderate heat stress reduces photosynthetic capacity. Although there is little or no damage to the photosystem due to mild heat stress, moderate heat stress can reduce the photosynthetic rate to near zero (7-9).

Siwa Oasis locates in the Qattara Depression that spans the northwest of Egypt. Much of the depression sits below sea level at its deepest. It sits 133 m below sea level, making it the second-lowest point in Africa. Steep slopes bound it to the Northside and the south and west it grades into the Great Sand Sea in the Sahara. The Oasis is promised newly reclaimed lands in Egypt as numerous economic plants successfully cultivated there. *H. sabdariffa* L. is a valuable crop expansively farmed at Siwa Oasis and is considered a good source of income for local farmers and investors. However, its yield quantity and quality traits were negatively affected and declined because of increasing temperature in the last decade; the absolute maximum temperature reached 50 ˚C during summer (10-13).

The use of plant biostimulants in agriculture in newly reclaimed lands is of great importance. Plant biostimulants are substances and materials, except nutrients and pesticides, which, when applied to plant, seeds, or growing substrates in specific formulations, can modify the physiological processes of plants in a way that provides potential benefits to growth, development and stress responses (14). Based on the preceding literature, the biostimulants such as salicylic acid and blue green algae extract of *Spirulina platensis* are observed to lessen heat stress on plants and contribute to rising yield (15-20).

Blue green algae, also called cyanobacteria, any of a large, heterogeneous group of prokaryotic, principally photosynthetic organisms. Cyanobacteria contain chlorophyll, a green pigment. In addition, they have the blue pigment phycobilin. The combination of phycobilin and chlorophyll produces the characteristic blue green color from which these organisms derive their famous name. They are widely distributed and are extremely common in freshwater. They are also abundantly represented in such habitats as tide pools, coral reefs and tidal spray zones. *Spirulina platensis* is a blue green alga rich in proteins, vitamins, minerals, carotenoids and antioxidants that help plants acclimate to heat stress and produce a good yield. Salicylic acid is a phenolic compound that functions as a plant growth regulator and boosts photosynthesis under heat stress by affecting different physiological functions and biochemical reactions (21-23).

Accordingly, the present research object investigates the effect of spray with different salicylic acid concentrations and the numbers of sprinkles with blue green algae extract to alleviate heat stress on plants, consequently developing yield aspects.

Materials and Methods

The work was conducted during 2018 and 2019 in the Experimental Farm of the Desert Research Center at Khamisa Village (29.21˚ N and 25.40˚ E), Siwa Oasis. The farm's soil and irrigation water properties were determined and recorded in Tables (1-3). The soil was sandy with medium content of salts (2624 ppm). Irrigation water was of moderate salinity (2931 ppm). Also, the meteorological data of this region are in Table (5).

Table 1. Mechanical analysis of soil of the experiment farm

Sand (%)	Silt (%)	Clay (%)	Soil texture
92.91	5.21	1.88	Sandy

Table 2. Chemical analysis of soil of the experiment farm

pH	E.C. (ppm)	O.M. (%)	Soluble anions (meq/l)				Soluble cations (meq/l)			
			CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.50	2624.00	0.51	-	3.61	31.32	6.10	8.61	7.50	0.22	24.70

Table 3. Chemical analysis of well water of the experiment farm

pH	E.C. (ppm)	Soluble anions (meq/l)				Soluble cations (meq/l)			
		CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.20	2931.00	-	1.79	33.90	13.86	11.58	11.52	25.64	0.81

The present experiment was executed under open field conditions. Seeds of *H. sabdariffa* L. (dark red variety) were sown on March 11th in both seasons at drip irrigation lines spaced 0.75 m apart and 0.50 m within droppers. Thinning was done at suitable growth, leaving 2 plants per hill (53333 plants per ha). All achievable field processes such as fertilization, irrigation, and pest control were recommendations throughout the good agriculture practices (24-26).

The arrangement of the investigation was a split-plot design with three replicates. Foliar spraying with salicylic acid was used at three concentrations 0, 500 and 1000 mg/l and presented in the main plots, while the used number of sprays with blue green algae extract was as 0, 1 and 2 times through the season and allocated to sub-plots. Provided salicylic acid was from El-Gomhouria Company for Medicines and Medical Supplies, Cairo, Egypt. Spraying with salicylic acid was done after 80 days of the sowing date. Blue green algae extract of *Spirulina platensis* strain was obtained from Algae Production Unit, National Research Center, Cairo, Egypt. The applied algae extract was at the concentration of 5 ml/l water. The algae extract analysis is presented in Table (4). Plants were sprayed with

Table 4. Chemical analysis of *Spirulina platensis* extract

N (%)	P (%)	K_2O (%)	Fe (%)	Mn (%)	Zn (%)	Cu (%)	Cytokinin (mg/l)	Gibberellic acid (mg/l)	Auxin (mg/l)
4.30	2.15	0.10	2.11	3.04	2.42	0.75	720.00	2729.10	531.58

Table 5. Means of the meteorological data of the Oasis during the seasons of 2018 and 2019

Month		Air temperature (ºC)			Humidity (%)	Precipitation (mm)	Wind (km/h)
		Max.	Min.	Average			
Winter season	22 to 31 December	19.20	10.00	14.60	65.00	0.00	15.33
	January	21.00	7.00	14.00	53.50	1.50	21.24
	February	25.50	7.00	16.25	50.50	2.10	17.98
	1 to 19 March	31.00	8.50	19.75	38.70	0.20	19.85
Mean		24.18	8.13	16.15	51.93	0.95	18.60
Spring season	20 to 31 March	30.00	9.00	19.50	46.33	0.00	23.23
	April	34.50	10.00	22.25	47.50	0.00	18.99
	May	41.50	13.50	27.50	34.50	0.00	17.82
	1 to 20 June	33.00	18.50	25.75	35.19	0.00	19.58
Mean		34.75	12.75	23.75	40.88	0.00	19.91
Summer season	21 to 30 June	38.00	21.00	29.50	38.01	0.00	14.45
	July	39.00	23.00	31.00	42.00	0.00	18.73
	August	42.00	24.00	33.00	42.50	0.00	16.34
	1 to 22 September	38.00	22.00	30.00	50.93	0.00	19.31
Mean		39.25	22.50	30.88	43.36	0.00	17.21
Autumn season	23 to 30 September	34.00	19.00	26.50	46.57	0.00	13.89
	October	32.50	14.50	23.50	48.50	0.00	18.34
	November	28.50	11.00	19.75	49.00	0.00	18.44
	1 to 21 December	24.00	6.00	15.00	58.34	0.00	25.11
Mean		29.75	12.63	21.19	50.60	0.00	18.95

algae extract after 80 days in the case of spraying once or spraying twice after 80 and 110 days of planting date. Sprinkling with the previous solutions was carried out on leaves of the plant until the surface run-off.

Harvesting of plants was conducted on November 8th by cutting the aerial parts above the soil surface. The taken growth and yield measurements were as follows: plant height (cm), dry weight/plant (g), number of fruits/plant, dry weight of sepals/plant (g), dry yield of sepals/hectare (kg) and dry yield of seeds/hectare (kg). The sepals were separated manually from their fruits and removed any foreign materials. The dry matter content was determined by drying at 70 °C up to constancy. The attained chemical analyses included:- determination of chlorophylls A, B and carotenoids (mg/g) in leaves (27) and anthocyanin content (mg/100 g) in sepals (28, 29). Manner of sampling ensured its representative of an entire lot of the raw material. The two-way ANOVA statistically tested all data according to (30). L.S.D examined the differences between means at 0.05.

Results and Discussion

Effect of salicylic acid concentrations

Tables (6, 7) displayed the influence of spray with different salicylic acid concentrations on growth traits. Significant increments in plant height and dry weight/plant were found by increasing salicylic acid concentration to 500 mg/l. Then, there was a decline in these estimates by increasing the solution concentration up to 1000 mg/l. In this concern, the mean values in the first season of using 500 mg/l concentration were 120.67 cm and 195.17 g; the mean values in the second season of the same concentration were 114.00 cm and 157.84 g for plant height and dry weight/plant respectively. So, based on these data, the unsprayed plants under heat stress conditions gave the lowest dry weight per plant, which could lower the fiber yield and using salicylic acid at 500 mg/l improved this lousy effect.

The effects of spraying with various salicylic acid concentrations on fruit yield attributes were shown in Tables (8-11). Increasing salicylic acid concentration to 500 mg/l resulted in significant increases in all fruit metrics. Then, as the solution concentration was increased to 1000 mg/l, these estimations began to drop. In this regard, for 500 mg/l, the mean values for number of fruits/plant, dry weight of sepals/plant, dry yield of sepals/ha and dry yield of seeds/ha in the first season were 54.80 fruits, 13.56 g, 723.02 kg and 937.99 kg; the mean values in the second season were 45.88 fruits, 12.26 g, 653.68 kg and 781.23 kg. So, based on the data as mentioned, the control plants under high-temperature circumstances showed the lowest fruit yield characteristics, which could reduce the sepals and seeds yield. Spraying salicylic acid at 500 mg/l improved this adverse effect.

Similarly, the pigment contents in the leaves and sepals were increased by increasing the salicylic acid concentration from 0 to 500 mg/l and were decreased when the concentration increased to 1000 mg/l (Tables 12-15). These findings in the first season were 1.40, 0.57, 0.39 mg/g and 438.61 mg/100 g; in the second season were 1.33, 0.43, 0.28 mg/g and 430.38 mg/100 g for chlorophylls A, chlorophylls B, carotenoids contents in leaves and anthocyanin content in sepals, in that order. In comparison, the control treatment included the lowest pigments.

The enhancement role of salicylic acid at the concentration of 500 mg/l on plants under heat stress was in agreement with the findings of numerous investigators. On grape, spraying of salicylic acid improved photosynthesis by alleviating photosystem II damage under heat stress. Salicylic acid also improved thermo tolerance through increasing photosynthetic capability and adjusting the distribution of assimilates (31). On wheat, salicylic acid interacted with proline metabolism and ethylene formation to alleviate the adverse effects of heat stress on photosynthesis (32). The negative impacts of heat stress on tomatoes could be suppressed by pretreated salicylic acid through increased photosynthesis activity and antioxidant enzymes functions (33). On ornamental pepper seedlings grown under high-temperature stress, spraying salicylic acid alleviated significantly yellow leaves and dwarf-

Table 6. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on plant height (cm)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	105.14 g	108.55 gi	122.76 l	112.15 a	96.56 g	101.88 i	118.76 l	105.73 a
500	111.88 i	121.31 hl	128.81 j	120.67 b	103.88 i	114.31 hl	123.81 j	114.00 b
1000	97.85 k	106.52 g	117.83 h	107.40 c	90.89 k	99.52 gi	112.76 h	101.06 c
Mean	104.96 d	112.13 e	123.13 f		97.11 d	105.24 e	118.44 f	
LSD at 0.05								
Salicylic acid	2.69				2.68			
Algae extract	2.70				2.53			
Interaction	4.66				4.64			

Means with the same letter are not significantly different at 5% level of probability

Table 7. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on dry weight/plant (g)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	89.31 g	130.69 im	219.50 k	146.50 a	72.64 g	110.69 im	169.50 k	117.61 a
500	144.21 m	184.90 j	256.41 l	195.17 b	122.21 m	149.90 j	201.41 l	157.84 b
1000	75.79 g	112.27 h	198.81 j	128.96 c	60.66 g	89.27 h	158.81 jk	102.91 c
Mean	103.10 d	142.62 e	224.91 f		85.17 d	116.62 e	176.57 f	
LSD at 0.05								
Salicylic acid	8.49				8.34			
Algae extract	8.52				8.40			
Interaction	14.70				14.45			

Means with the same letter are not significantly different at 5% level of probability

Table 8. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on number of fruits/plant

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	31.89 gh	42.87 mi	60.53 k	45.10 a	24.00 gh	30.00 mi	54.53 l	36.18 a
500	44.17 mj	50.19 j	70.03 l	54.80 b	32.17 m	43.44 j	62.03 n	45.88 b
1000	30.08 g	37.17 hi	56.89 k	41.38 c	20.42 g	27.17 hi	48.89 k	32.16 c
Mean	35.38 d	43.41 e	62.48 f		25.53 d	33.54 e	55.15 f	
LSD at 0.05								
Salicylic acid	3.70				2.88			
Algae extract	3.70				2.80			
Interaction	6.45				4.99			

Means with the same letter are not significantly different at 5% level of probability

Table 9. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on dry weight of sepals/plant (g)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	9.67 h	11.01 i	13.67 k	11.45 a	8.95 h	10.01 i	12.00 j	10.32 a
500	11.97 ij	12.80 jk	15.90 l	13.56 b	10.31 i	12.46 j	14.00 k	12.26 b
1000	8.20 g	9.67 h	12.95 jk	10.27 c	6.20 g	8.00 h	11.09 ij	8.43 c
Mean	9.47 d	11.16 e	14.17 f		8.49 d	10.16 e	12.36 f	
LSD at 0.05								
Salicylic acid		0.69				0.75		
Algae extract		0.63				0.80		
Interaction		1.20				1.29		

Means with the same letter are not significantly different at 5% level of probability

Table 10. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on dry yield of sepals/ hectare (kg)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	515.73 ^{h}	587.20 ^{i}	729.06 ^{k}	610.66 ^{a}	477.33 ^{h}	533.86 ^{i}	640.00 ^{j}	550.40 ^{a}
500	638.40 ^{ij}	682.66 ^{jk}	847.99 ^{l}	723.02 ^{b}	549.86 ^{i}	664.53 ^{j}	746.66 ^{k}	653.68 ^{b}
1000	437.33 ^{g}	515.73 ^{h}	690.66 ^{jk}	547.91 ^{c}	330.66 ^{g}	426.66 ^{h}	591.46 ^{ij}	449.59 ^{c}
Mean	530.49 ^{d}	595.20 ^{e}	755.90 ^{f}		452.62 ^{d}	541.68 ^{e}	659.37 ^{f}	
LSD at 0.05								
Salicylic acid	38.29				41.20			
Algae extract	38.19				41.30			
Interaction	66.12				71.36			

Means with the same letter are not significantly different at 5% level of probability

Table 11. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on dry yield of seeds/ hectare (kg)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	558.81 ^{g}	653.98 ^{hi}	1088.85 ^{l}	767.21 ^{a}	405.55 ^{gh}	497.95 ^{ij}	968.78 ^{l}	624.09 ^{a}
500	712.62 ^{i}	805.02 ^{j}	1296.33 ^{m}	937.99 ^{b}	546.64 ^{j}	666.70 ^{k}	1130.35 ^{m}	781.23 ^{b}
1000	535.57 ^{g}	597.00 ^{gh}	968.78 ^{k}	700.45 ^{c}	341.92 ^{g}	467.52 ^{hi}	911.26 ^{l}	573.57 ^{c}
Mean	602.33 ^{d}	685.33 ^{e}	1117.99 ^{f}		431.37 ^{d}	544.06 ^{e}	1003.46 ^{f}	
LSD at 0.05								
Salicylic acid	36.14				38.01			
Algae extract	35.82				38.04			
Interaction	62.57				65.83			

Means with the same letter are not significantly different at 5% level of probability

ing. Salicylic acid maintained high root vigor, inhibited water loss, and maintained the integrity of cell structure by regulating osmotic substance content. Salicylic acid decreased the production of reactive oxygen species, increased the activity of protective enzymes, and the concentration of non-enzymatic reactive oxygen species scavengers (20). Also, our work was in the same line with the literature by (34-38).

On the other side, the highest concentration of salicylic acid at 1000 mg/l negatively affected plants under stress conditions. It reduced shoot growth, sepals and seed yield per plant and hectare. These results coincided with several researchers. Stomatal closing by the overdose salicylic acid concentration was related to the decreased photosynthetic activity (39). A decline in the production of Egyptian clover when using the over-dose salicylic acid was found (40). Also, (41) mentioned that salicylic acid might be a stress factor and negatively influence various physiological processes. Its mode of action depended greatly on several factors, such as plant species, environmental conditions (light, temperature etc.) and concentration. Controlled salicylic acid levels were essential in plants for optimal photosynthetic performance and acclimating to changing environmental stimuli.

Effect of the number of sprays with Spirulina extract

Data presented in Tables (6, 7) showed the number of sprays with Spirulina extract on growth. Results revealed that foliar spray with extract twice through the season was superior to one addition and control as it gave the significant highest values. These values in the first season were 123.13 cm and 224.91 g; these values in the second season were 118.44 cm and 176.57 g, for plant height and dry weight/plant, in turn. So, according to these data, the two sprays promoted dry weight and consequently expected fiber yield than other applications.

The impact of the number of sprays with *Spirulina* extract on fruit yield was shown in Tables (8-11). The results showed that foliar spraying with extract twice during the season was preferable to one addition and control since it produced the most excellent significant values. In the first season, these means were 62.48 fruits, 14.17 g, 755.90 kg, and 1117.99 kg; in the second season, these means were 55.15 fruits, 12.36 g, 659.37 kg, and 1003.46 kg, for number of fruits/plant, dry weight of sepals/plant, yield of sepals/ha and yield of seeds/hectare, subsequently. Thus using blue green algae extract two times enhanced the fruit characters under stress.

Likewise, foliar spray with blue green algae extract twice significantly raised the pigment contents in leaves and sepals than in other treatments (Tables 12-15). These detections in the first season were 1.52, 0.57, 0.39 mg/g, and 447.17 mg/100 g; these evaluations in the second season were 1.44, 0.46, 0.29 mg/g, and 434.13 mg/100 g for chlorophylls A, chlorophylls B, carotenoids contents in leaves and anthocyanin content in sepals, correspondingly.

Table 12. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on chlorophylls A content in leaves (mg/g)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	1.07 ^{g}	1.16 ^{i}	1.51 ^{m}	1.25 ^{a}	1.00 ^{g}	1.11 ^{j}	1.46 ^{n}	1.19 ^{a}
500	1.25 ^{j}	1.35 ^{k}	1.61 ^{l}	1.40 ^{b}	1.18 ^{k}	1.30 ^{l}	1.52 ^{p}	1.33 ^{b}
1000	1.07 ^{g}	1.13 ^{h}	1.43 ^{n}	1.21 ^{c}	0.98 ^{h}	1.04 ^{i}	1.34 ^{m}	1.12 ^{c}
Mean	1.13 ^{d}	1.21 ^{e}	1.52 ^{f}		1.05 ^{d}	1.15 ^{e}	1.44 ^{f}	
LSD at 0.05								
Salicylic acid	0.01				0.01			
Algae extract	0.01				0.01			
Interaction	0.01				0.02			

Means with the same letter are not significantly different at 5% level of probability

Table 13. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on chlorophylls B content in leaves (mg/g)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	0.34 ^{h}	0.43 ^{j}	0.58 ^{l}	0.45 ^{a}	0.24 ^{h}	0.28 ^{i}	0.45 ^{l}	0.32 ^{a}
500	0.51 ^{k}	0.59 ^{m}	0.62 ^{n}	0.57 ^{b}	0.37 ^{j}	0.41 ^{k}	0.52 ^{m}	0.43 ^{b}
1000	0.29 ^{g}	0.38 ^{i}	0.51 ^{k}	0.39 ^{c}	0.19 ^{g}	0.24 ^{h}	0.41 ^{k}	0.28 ^{c}
Mean	0.38 ^{d}	0.47 ^{e}	0.57 ^{f}		0.27 ^{d}	0.31 ^{e}	0.46 ^{f}	
LSD at 0.05								
Salicylic acid	0.01				0.03			
Algae extract	0.01				0.03			
Interaction	0.02				0.04			

Means with the same letter are not significantly different at 5% level of probability

Table 14. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on carotenoids content in leaves (mg/g)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	0.37 ^{f}	0.37 ^{f}	0.39 ^{h}	0.38 ^{a}	0.19 ^{h}	0.21 ^{hi}	0.30 ^{k}	0.23 ^{a}
500	0.38 ^{g}	0.38 ^{g}	0.41 ^{i}	0.39 ^{b}	0.23 ^{ij}	0.29 ^{k}	0.31 ^{k}	0.28 ^{b}
1000	0.37 ^{f}	0.37 ^{f}	0.38 ^{g}	0.37 ^{c}	0.16 ^{g}	0.19 ^{h}	0.25 ^{j}	0.20 ^{c}
Mean	0.37 ^{d}	0.37 ^{d}	0.39 ^{e}		0.19 ^{d}	0.23 ^{e}	0.29 ^{f}	
LSD at 0.05								
Salicylic acid	0.01				0.02			
Algae extract	0.01				0.02			
Interaction	0.01				0.03			

Means with the same letter are not significantly different at 5% level of probability

Table 15. Effect of spray with different salicylic acid concentrations, number of sprays with blue green algae extract, and their interaction on anthocyanin content in sepals (mg/100g)

Treatments	1st season				2nd season			
Salicylic acid	Algae extract				Algae extract			
	0	1	2	Mean	0	1	2	Mean
0	403.20 ^{g}	421.80 ^{hi}	444.33 ^{k}	423.11 ^{a}	398.38 ^{g}	417.41 ^{i}	430.48 ^{l}	415.42 ^{a}
500	426.71 ^{ij}	431.47 ^{j}	457.64 ^{l}	438.61 ^{b}	420.76 ^{j}	424.63 ^{k}	445.74 ^{m}	430.38 ^{b}
1000	400.04 ^{g}	420.43 ^{h}	439.55 ^{k}	420.01 ^{c}	398.07 ^{g}	411.87 ^{h}	426.16 ^{k}	412.03 ^{c}
Mean	409.98 ^{d}	424.57 ^{e}	447.17 ^{f}		405.74 ^{d}	417.97 ^{e}	434.13 ^{f}	
LSD at 0.05								
Salicylic acid	2.89				1.66			
Algae extract	2.89				1.66			
Interaction	5.01				2.87			

Means with the same letter are not significantly different at 5% level of probability

But applications of algae extract one time and control plants gave lower pigment concentrations.

The promoted influence of spray with *Spirulina* extract on plants could be attributed to its high concentration of macro and microelements, besides amino acids. It also had natural carotene and xanthophyll phytopigments, which were a natural source of vitamin B-12. In addition, high levels of different plant hormones such as gibberellic acid, auxins and cytokinins were vital to increase production and increase plant tolerance to abiotic stresses (Table 4). For these reasons, spraying with *Spirulina* extract twice was better than once and the control. The noticed stimulant effect of algae extract on the crops was consistent with (17) on marjoram, (18) on lemongrass, (42) on some plants of edible amaranth, phaseolus and tomato, (43) on sugar beet, (44) on wheat, (45) on red roomy grapevines and (46) on broad bean.

Effect of the interaction between salicylic acid concentrations and number of sprays with Spirulina extract

Tables (6-7) showed the interaction among treatments on growth characters. The significantly maximum parameters were from spraying with salicylic acid at the concentration of 500 mg/l plus spraying with *Spirulina* extract two times through the season. These values in the first season were 128.81 cm and 256.41 g; these records in the second season were 123.81 cm and 201.41 g for plant height and dry weight/plant respectively. On the contrary, any interaction between spraying with salicylic acid at the highest concentration (1000 mg/l) and *Spirulina* treatments (0, 1 and 2 sprays in season) lowered the growth.

The interaction between treatments on yield characters was shown in Tables (8-11). Spraying with salicylic acid at a concentration of 500 mg/l and spraying with *Spirulina* extract twice during the season, resulting in the most significant maximum parameters. These records for number of fruits/plant, dry weight of sepals/plant and hectare and dry yield of seeds/ha were 70.03 fruits, 15.90 g, 847.99 kg and 1296.33 kg in the first season and 62.03 fruits, 14.00 g, 746.66 kg and 1130.35 kg in the second season respectively. On the other hand, the interaction between spraying with salicylic acid at the highest dosage (1000 mg/l) and *Spirulina* treatments (0, 1 and 2 sprays in season) reduced sepals yield, as well as seed production.

As for the effect of interaction on pigments, it had the same trend. Data clearly showed that the treatment of spraying with salicylic acid at the concentration of 500 mg/l combined with the spraying of *Spirulina* extract two times through the season gave the significantly highest records (Tables 12-15). These values in the first season were 1.61, 0.62, 041 mg/g, and 457.64 mg/100 g. These records in the second season were 1.52, 0.52, 0.31 mg/g and 445.74mg/100 g for chlorophylls A, chlorophylls B, carotenoid contents in leaves and anthocyanin content in sepals, in order. While the combination of 1000 mg/l salicylic acid and algae extract treatments detected a decline in parameters.

Our results were in harmony with the investigation of (47) in Iraq on some *H. sabdariffa* L. varieties. They reported that spraying with salicylic acid and seaweed extract and their interactions gave the best results of active medical compounds. Furthermore, the work of (48) on Sewy date palms investigated the effect of spraying *Spirulina platensis* algae extract, salicylic acid and other materials on growth, nutritional status, yield and fruit quality under the sandy soil. They announced that an outstanding promotion was observed on yield and fruit quality due to spraying the palms with a mixture of *Spirulina platensis* algae extract, salicylic acid and other materials. Also, the output of our experiment agreed with the findings of (49). They studied the foliar application of *Spirulina platensis* algae and salicylic acid on flowering (%), growth, yield and fruit quality of Koroneiki olive trees grown under the sandy soil. Their results showed that combination applications were more practical than using each material alone in this respect. Applying two sprays of a mixture of *Spirulina platensis* extract and salicylic acid gave the best yield and fruit quality results.

The Oasis had high summer temperatures in addition to saline groundwater. Thus, during summer, there was an increase in salt stress too which negatively affected crop. Based on proceeding data, the efficiency of the treatment (spraying with salicylic acid at the concentration of 500 mg/l plus spraying of *Spirulina* extract two times through season) was due to their combined enhancement role on plant physiological processes as described before, which boosted the growth, yield with rich secondary metabolites. The calyx production/hectare increases over control treatment ranged from 64.43 to 56.42 % for the first and second seasons respectively (Fig. 1) and (Fig. 2). In

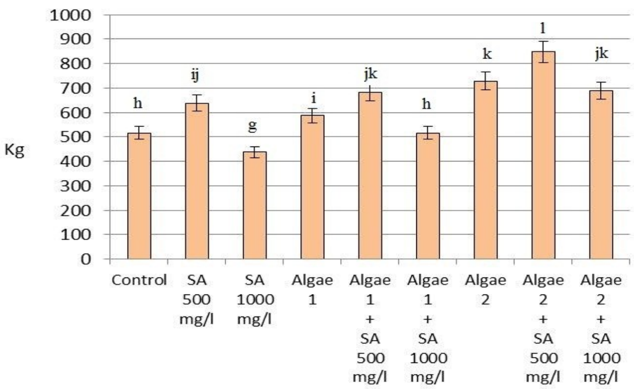

Fig. 1. Effect of interaction within treatments on dry yield of sepals in the 1st season (SA: salicylic acid, Algae 1: a spray of algae extract one time, Algae 2: a spray of algae extract two times).

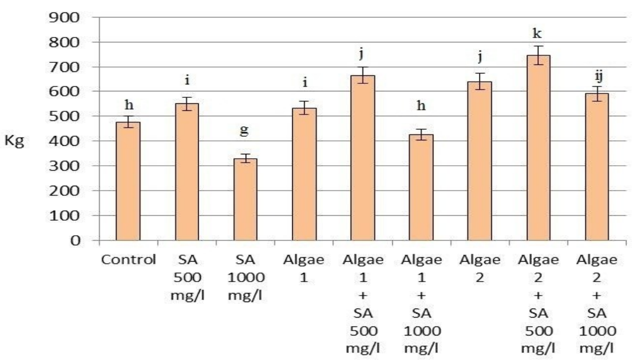

Fig. 2. Effect of interaction within treatments on dry yield of sepals in the 2nd season (SA: salicylic acid, Algae 1: a spray of algae extract one time, Algae 2: a spray of algae extract two times)

turn, the increases in sepal's anthocyanin content as a quality parameter were from 13.50 to 11.89 % over control for the first and second seasons, apart. Thus, we could endorse this treatment due to high economic return.

Conclusion

We recommend foliar spray of *H. sabdariffa* L. plants with salicylic acid at the concentration of 500 mg/l after 80 days of sowing date, besides foliar spray with *Spirulina platensis* algae extract two times through season after of 80 and 110 days with the concentration of 5 ml/l to have the highest yield under Siwa ecosystem.

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Authors contributions

All authors contributed equally. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Al-Shoosh WGA. Chemical Composition of Some Roselle (*Hibiscus sabdariffa*) Genotypes. M.Sc. Thesis, Khartoum University, Sudan; 1997.
2. Mohamed BB, Sulaiman AA, Dahab AA. Roselle (*Hibiscus sabdariffa* L.) in Sudan, cultivation and their uses. Bull Environ Pharmacol Life Sci. 2012;1(6):..54-48
3. Abdelfattah MS, Badr SEA, Lotfy SA, Attia GH, Aref AM, Abdel Moneim AE, Kassab RB. Rutin and selenium co-administration reverse 3-nitropropionic acid-induced neurochemical and molecular impairments in a mouse model of Huntington's disease. Neurotox Res. 2020;37:77-92. https://doi.org/10.1007/s12640-019-00086-y
4. Akim A, Ling LC, Rahmat A, Zakaria ZA. Antioxidant and antiproliferative activities of roselle juice on Caov-3, MCF-7, MDA-MB -231 and HeLa cancer cell lines. Afr J Pharm Pharmacol. 2011;5 (7):957-65.
5. Badr SEA, Sakr DM, Mahfouz SA, Abdelfattah MS. Licorice (*Glycyrrhiza glabra* L.): Chemical composition and biological impacts. Res J Pharm Biol Chem Sci. 2013;4(3):606-21.
6. Badr SEA, Abdelfattah MS, El-Sayed SH, Abd El-Aziz ASE, Sakr DM. Evaluation of anticancer, antimycoplasmal activities and chemical composition of guar (*Cyamopsis tetragonoloba*) seeds extract. Res J Pharm Biol Chem Sci. 2014;5:413-23.
7. Berry JA, Björkman O. Photosynthetic response and adaptation to temperature in higher plants. Annu Rev Plant Physiol Plant Mol Bio. 1980;31:491-543. https://doi.org/10.1146/annurev.pp.31.060180.002423
8. Guilioni LAC, Wery JB, Lecoeur JA. High temperature and water deficit may reduce seed number in field pea purely by decreasing plant growth rate. Funct Plant Biol. 2003;30: 1151-64. https://doi.org/10.1071/FP03105
9. Munoz R, Quiles MJ. Water deficit and heat affect the tolerance to high illumination in Hibiscus plants. Int J Mol Sci. 2013;14:5432-44. https://doi.org/10.3390/ijms14035432
10. Hammad MB, Hosny MA. Siwa Oasis, a neglected paradise. 3rd IRT International Scientific Conference: Integrated Relational Tourism Territories and Development in the Mediterranean Area. Conference proceedings. 2009; (2)765-72.
11. El Shishtawy AM. Impact of natural environment on the development of remote areas: Siwa Oasis, Western Desert, Egypt. International Conference on Geosciences and Geophysics; 2016 October 06-07; Orlando, USA.
12. FAO.Siwa Oasis - Egypt [Internet]. 2016. Available from: http://www.fao.org/3/bp825e/bp825e.pdf
13. Bullough F. The wet with the dry: The geology of Siwa Oasis [Internet]. 2014. Available from: https://blogs.egu.eu/network/4degrees/2014/03/10/the-wet-with-the-dry-the-geology-of-siwa-oasis/
14. Rouphael Y, Colla G. Biostimulants in Agriculture. Frontiers Media SA. 2020; PMID: 32117379. https://doi.org/10.3389/fpls.2020.00040
15. Gharib FA. Effect of salicylic acid on the growth, metabolic activities and oil content of basil and marjoram. Int J Agric Biol. 2006;8(4):485-92.
16. Kaur P, Ghai N, Sangha MK. Induction of thermotolerance through heat acclimation and salicylic acid in *Brassica* species. Afr J Biotechnol. 2009;8(4):619-25.
17. Abdalla MA, Hendi DMG. Effect of cyanobacterial foliar application and different levels of NPK fertilizer on growth, chemical composition and antioxidant activity of *Origanum majorana* L. Scientific J. Flowers and Ornamental Plants. 2014;1(2):171-86. https://doi.org/10.21608/sjfop.2014.4113
18. El-Mahrouk EM, Abido AI, Radwan FI, Hamed ES, El-Nagar EE. Vegetative growth and essential oil productivity of lemongrass (*Cymbopogon citratus*) as affected by NPK and some growth stimulators. Int J Bot Stud. 2018;3(6):48-55.
19. Lin KH, Huang SB, Wu CW, Chang YS. Effects of salicylic acid and calcium chloride on heat tolerance of poinsettia. HortScience. 2019;54(3):499-504. https://doi.org/10.21273/HORTSCI13566-18
20. Zhang Z, Lan M, Han X, Wu J, Wang-Pruski G. Response of ornamental pepper to high-temperature stress and role of exogenous salicylic acid in mitigating high temperature. J Plant Growth Regul. 2020;39:133-46. https://doi.org/10.1007/s00344-019-09969-y
21. Raskin I (1992). Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol. 1992;43:493-63. https://doi.org/10.1146/annurev.pp.43.060192.002255
22. Shawky SM, Mostafa SSM, Abd El-Alla AM. Efficacy of algae, azolla and compost extract in controlling rook knot nematode and its reflection on cucumber growth. Bull Fac Agric Univ Cairo. 2009;60:443-59. https://doi.org/10.21608/ejarc.2009.215929
23. Sheffer CP. blue-green algae organism. [Internet]. https://www.britannica.com/science/blue-green-algae
24. Badawi AMA 2000. Effect of Some Agricultural Treatments on Growth and Active Ingredients in Roselle Plants in North Sinai. Ph.D. Thesis, Zagazig University, Egypt. 2000.
25. World Health Organization. WHO Guidelines on Good Agricultural and Collection Practices [GACP] for Medicinal Plants [Internet]. 2003. Available from: http://apps.who.int/iris/bitstream/handle/10665/42783/9241546271.pdf;jsessionid=0EF9CA8671A49BDDE765F5BF589E1B06?sequence=1

26. Heron B. Good Agricultural and Collection Practices for Medicinal Plants. FAO. 2010.

27. Cherry JH. Molecular Biology of Plants (A text manual). New York: Columbia Univ. Press; 1973.

28. Fuleki T, Francis FJ. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. J Food Sci. 1968;33:72-77. https://doi.org/10.1111/j.1365-2621.1968.tb00887.x

29. Francis FJ. Anthocyanins and betalains composition: composition and applications. CFW Plex. 2000;45:208-13.

30. Snedecor GW, Cochran WG. Statistical Methods. Iowa, U.S.A: The Iowa State Univ. Press; 1982.

31. Wang LJ, Li SH. The effects of salicylic acid on distribution of ^{14}C-assimilation and photosynthesis in young grape plants under heat stress. Acta Hortic. 2007;738:779-85. https://doi.org/10.17660/ActaHortic.2007.738.104

32. Khan MIR, Iqbal N, Masood A, Per TS, Khan NA. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signal Behav. 2013; 8(11):e26374-1 to e26374-10. https://doi.org/10.4161/psb.26374

33. Jahan MS, Wang Y, Shu S, Zhong M, Chen Z, Wu J, Sun J, Guo S. Exogenous salicylic acid increases the heat tolerance in Tomato (*Solanum lycopersicum* L.) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. Sci Hortic. 2019;247:421-29. https://doi.org/10.1016/j.scienta.2018.12.047

34. Al-Jeboori KD, Al-Mharib MZK, Hamdan AQ, Mahmood AH. Effect of irrigation intervals and foliar of salicylic acid on growth and yield of potato. Iraqi J Agric Sci. 2017;48(1):242-47. https://doi.org/10.36103/ijas.v48i1.440

35. Khattab EA, El-Housini EA, Khedr HH. Effect of some antioxidants (ascorbic acid, proline, and salic acid) on jojoba plants under circumstance of Sinai. Iraqi J Agric Sci. 2019; 50(4):1086-93. https://doi.org/10.36103/ijas.v50i4.753

36. Mohammed AA, Abbas JM, Al-Baldawi MHK. Effect of salicylic acid spraying on yield and it's components of linseed cultivars. Iraqi J Agric Sci. 2020;51(2):585-91. https://doi.org/10.36103/ijas.v51i2.985

37. Al-Atrushy ShMM. Effect of foliar application of zink and salicylic acid on vegetative growth and yield characteristics of Halawani grape cultivar (*Vitis vinifera* L.). Iraqi J Agric Sci. 2021;52(4):989-98. https://doi.org/10.36103/ijas.v52i4.1410

38. Rad ZM, Nourafcan H, Mohebalipour N, Assadi A, Jamshidi S. Effect of Salicylic acid foliar application on phytochemical composition, antioxidant and antimicrobial activity of *Silybum marianum*. Iraqi J Agric Sci. 2021;52(1):63-69. https://doi.org/10.36103/ijas.v52i1.1236

39. Joon-Sang L. The mechanism of stomatal closing by salicylic acid in *Commelina communis* L. J Plant Biol. 1998;41:102-97. https://doi.org/10.1007/BF03030395

40. Kumar B, Singh Y, Ram H, Sarlach RS. Enhancing seed yield and quality of Egyptian clover (*Trifolium alexandrinum* L.) with foliar application of bio-regulators. Field Crops Res. 2013;146:25-30. https://doi.org/10.1016/j.fcr.2013.03.004

41. Janda T, Gondor OK, Yordanova R, Szalai G, Pál M. Salicylic acid and photosynthesis: signaling and effects. Acta Physiol Plant. 2014;36:2537-46. https://doi.org/10.1007/s11738-014-1620-y

42. Anitha L, Bramari GS, Kalpana P. Effect of supplementation of *Spirulina platensis* to enhance the zinc status in plants of *Amaranthus gangeticus*, *Phaseolus aureus* and tomato. Adv Biosci Biotechnol. 2016;7:289-99. https://doi.org/10.4236/abb.2016.76027

43. Abbas MS, Dewdar MH, Gaber EI, AbdEl-Aleem HA. Impact of boron foliar application on quantity and quality traits of sugar beet (*Beta vulgaris* L.) in Egypt. Res J Pharma Biol Chem Sci. 2014;5(5):143-51.

44. Abd El-Rheem Kh M, Zaghloul Sahar M, Essa Entsar M. The stimulant effect of the *Spirulina* algae under low levels of nitrogen fertilization on wheat plants grown in sandy soils. Int J Chemtech Res. 2015;8(12):87-91.

45. Abou-Zaid Eman AA, Mokhtar MS. Growth, yield and berries quality in Red Roomy grapevines improved under different foliar application of *Spirulina* algae, zinc and boron. Middle East J Agric Res. 2019;8(2):654-61.

46. Selem Eman E. Physiological effects of *Spirulina platensis* in salt stressed *Vicia faba* L. Plants. Egypt J Bot. 2019;59(1):185-94. https://doi.org/10.21608/EJBO.2018.3836.1178

47. Hasson AS, Ramadan EL, Hussain MH. Effect of salicylic acid and seaweed extract in the content of sepals of some active medical compounds for several varieties of roselle *Hibiscus Sabdariffa* L. Int J STEM Educ. 2017;4(4):7068-73.

48. Hussien MA. Productive performance of Sewy date palms in relation to spraying *Spirulina platensis* algae, plant compost tea, salicylic acid and tocopherol. N Y Sci J. 2017;10(7):126-35. https://doi:10.7537/marsnys100717.17.

49. Hussein Esraa ME, Gad El- Kareem MR. Response of koroneiki olive trees to foliar application of *Spirulina platensis* algae and salicylic acid. SVUIJAS. 2021;3(3):245-54. https://doi.org/10.21608/svuijas.2021.79063.1114

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