

RESEARCH PAPER

The influence of plant growth regulators on phytochemical components in the leaves and calyxes of Roselle (*Hibiscus sabdariffa* L.)

Media I. MuhammedAmin¹, Sawsan M. S. Ali Kanimarani²

¹Horticulture department, College of Agricultural Science and Engineering, Salahaddin University-Erbil, Kurdistan Region, Iraq

²Horticulture department, College of Agricultural Science and Engineering, Salahaddin University-Erbil, Kurdistan Region, Iraq

ABSTRACT:

A study was conducted at the open field of Zanco Village, Erbil-Iraq in 2018 to determine the effect of foliar application of some plant growth regulators (IAA, BA and GA₃) on phytochemical components in the leaves and calyxes of Roselle. The investigation was performed as factorial experiment under randomized complete block design with three replications, growth regulators were foliar applied alone or combined treatments as: control, IAA (100 mg.l⁻¹), BA (150 mg.l⁻¹), GA₃ (150 mg.l⁻¹), IAA (100 mg.l⁻¹) + BA (150 mg.l⁻¹), IAA (100 mg.l⁻¹) + GA₃ (150 mg.l⁻¹), BA (150 mg.l⁻¹) + GA₃ (150 mg.l⁻¹) and IAA (100 mg.l⁻¹) + BA (150 mg.l⁻¹) + GA₃ (150 mg.l⁻¹). Parameters under study were: Moisture content (%) in the stem and Chlorophyll (spad) (%), Protein (%), Total soluble solid (%), Total flavonoids (mg. g⁻¹), Anthocyanin (mg.kg⁻¹), Ascorbic acid (vitamin C) mg.kg⁻¹, Genistein (mg.kg⁻¹), Hesperetin (mg.kg⁻¹) and Myrecetin (mg.kg⁻¹), were recorded in the leaves and calyxes of Roselle. Results showed that the highest levels of plant growth regulators in single and combined forms give the highest values of the above-mentioned parameters except treatment 100 mg.l⁻¹ IAA caused significant difference only in total flavonoids (mg.g⁻¹).

KEY WORDS: *Hibiscus sabdariffa* L., plant growth regulators; phytochemical components; Leaves and Calyxes.

DOI: <http://dx.doi.org/10.21271/ZJPAS.32.3.20>

ZJPAS (2020) , 32(3);193-199

1.INTRODUCTION

Roselle *Hibiscus sabdariffa* L. is an annual herb belongs to Malvaceae family and cultivated mainly for its leaves, stems, seeds and fruits (Fasoyiro *et al.* 2005). It is an important medicinal plant which is used for curing various degenerative diseases like hypertensions, cancer and inflammatory of liver and kidney (Riaz and Chopra,2018). The bright red and fleshy cup-shaped fruits are the most important part of Roselle plants that can be managed into food and beverages, pharmaceuticals and cosmetic products (Mohamad *et al.* 2011). Plant growth regulators considered as a new generation of agrochemicals that affects plant growth physiology and influences a plant's natural rhythm when added in small quantities, as stated earlier, in certain physiological processes pursuit in plant systems, growth regulators contribute in dynamic utilization of metabolites (Antony *et al.*, 2003).

* Corresponding Author:

Media I. MohammedAmin

E-mail: media.mohammedamin@su.edu.krd or sussan_ms@yahoo.com

Article History:

Received: 22/10/2019

Accepted: 15/12/2019

Published:15/06/2020

Among the plant growth regulators, Auxins are primary regulators of plant form. The auxin Indole 3-acetic acid (IAA) is a natural auxin found in the plants and present in a synthetic form, while the cytokinin Benzyladenine (BA) is a synthetic plant regulator (Bidwell, 1979 and Friml, 2003). BA is used to promote branching and increase flower set. Gibberellic acid (GA₃) is known as growth stimulators which mediate many reactions in plants, from germination of seeds to senescence (Mostafa and Abou Al-Hamd, 2011). Moreover, Kadiri *et al.* (1997) show that single and combined growth regulator treatments of 100 mg.l⁻¹ IAA, 100 mg.l⁻¹ GA₃ and 10% and 15% coconut milk significantly increased chlorophyll and vitamin C contents of Okra (*Abelmoschus esculentus* L.) and Roselle (*Hibiscus sabdariffa* L.) Also, Aycock *et al.* (1999) stated that the treatment with cytokinin for Gossypium plants showed a significant yield increases. However, Hassanein *et al.* (2005) noticed that the maximum increase of anthocyanin (mg.g⁻¹) in Roselle sepals had been registered in response to 100 mg.l⁻¹ of both GA₃ + BA. Mukhtar (2008) noticed that foliar application treatments with 100 mg.l⁻¹ IAA, 100 mg.l⁻¹ GA₃ and 15% coconut milk significantly increased total chlorophyll contents (mg.g⁻¹), vitamin C (µg/g) and protein (%) of Roselle. Hayssam *et al.* (2012) discovered that using of GA₃ (10⁻⁶ M) improve relative water content, Chlorophyll a, b, total Chlorophyll and anthocyanin by comparing to control, when they elaborated the influence of GA₃ on the growth and photosynthetic pigments of Roselle under salt stress in Saudi Arabia. Ramtin *et al.* (2015) showed that by spraying plants via benzyl adenine 50 µM (8.973%) on standard Carnation (*Dianthus caryophyllus* L.), it had more water content which is about two folds more than control (4.426%). Khandaker *et al.* (2018) showed that foliar spray of 60 mg.l⁻¹ GA₃ increased chlorophyll (spad) content (47.5) in Okra var. Singa 979, and the highest total soluble solids (TSS) content (2.47% Brix) were recorded for the 90 mg.l⁻¹ IAA treatment. Because of the few studies in Iraqi Kurdistan region about the effect of plant growth regulators on the phytochemical components of Roselle (*H. sabdariffa* L.) this study was conducted to theorize the impacts of some plant growth regulators (PGR) on the leaves and

calyxes on this of Roselle in Kurdistan environment.

2.MATERIALS AND METHODS

The experiment was carried out during May 22th to December 31th 2018 at Zanco Village open field, Erbil-Iraq to study the effect of foliar spraying of different growth regulators (Indole acetic acid, Benzyle adenine and Gibberillic acid) on chemical constituents of Roselle (*H. sabdariffa* L.). Several soil samples were taken from different locations of field depending on the depth of 0-30 cm (Estefan *et al.*, 2013), the soil analysis findings are shown in table (1). Table (2) displays the metrological data analyzed during the experimental phase.

Table (10) Some physical and chemical properties of the soil used in the study*

Properties	Field soil
pH	7.78
Electro conductivity (EC)	0.2 dS.m ⁻¹
Organic matter	0.01%
Total potassium (K ₂ O)	176 mg.l ⁻¹
Clay	25.4%
Silt	25.9%
Sand	48.7%
Soil Texture	Sandy Clay Loam

*Laboratory of Directorate of Research in Erbil/Soil and Laboratories Department.

Table (2) The metrological data during the study periods*

Months	Average Temperature C°		Average Relative Humidity %	Sum of Rain /mm
	Minimum	Maximum		
May	12.25	39.59	43.28	27.60
June	21.60	46.4	21.00	0.00
July	20.43	46.15	15.60	0.00
August	19.69	43.73	17.55	0.00
September	14.91	42.80	18.40	0.0
October	7.07	37.41	37.62	31.3
November	4.95	28.32	70.28	118.6
December	1.16	18.73	80.67	174.2

*Agriculture research center Erbil, Ministry of Kurdistan region.

2.1 Seed sowing and cultivation

The seeds of (*H. sabdariffa* L.) were gained from the research centre of agriculture, Ministry of Agriculture, Erbil- Iraq.

The seeds were dressed by Raxil fungicide (1.5 kg.ton⁻¹) three days before sowing; seeds were sown on May, 22th 2018 (3 seeds. hole⁻¹) with spacing of 40cm between holes and 50 cm between rows. The seedlings at the stage of 3 true leaves were thinned to one plant. hole⁻¹, leaving healthy and uniform seedlings, each plot contains 6 seedlings (Castro *et al.*, 2004, Ahmed *et al.*, 2011 and Gebremedin, 2015).

2.2 Plant growth regulators treatments

The Plant growth regulators(PGRs) IAA, BA (99.9% supplied by, Transhuman Technologies LTD, London, UK) and GA₃ (90% supplied by, dephyte, Germany). These two levels of each of PGRs (0 and 100 mg.l⁻¹) IAA and (0 and 150 mg.l⁻¹) were dissolved in a few drops of 1N sodium hydroxide (NaOH), (0 and 150 mg.l⁻¹) BA was dissolved in a few drops of 1N hydrochloric acid (HCl) (Pullaiah *et al.*, 2017).

2.3 The experiment's description

The experiment was considered a factorial in Randomized Complete Block Design with 3 blocks of 8 experimental units each (120×50 cm) representing single and combined growth regulator treatments, each unit contain 6 plants. The treatments were:

- 1- control (only distilled water)
- 2- IAA 100 mg.l⁻¹
- 3- BA 150 mg.l⁻¹
- 4- GA₃150 mg.l⁻¹
- 5- IAA (100 mg.l⁻¹) + BA (150 mg.l⁻¹)
- 6- IAA (100 mg.l⁻¹) + GA₃ (150 mg.l⁻¹)
- 7- BA (150 mg.l⁻¹) + GA₃ (150 mg.l⁻¹)
- 8- IAA (100 mg.l⁻¹) + BA (150 mg.l⁻¹) + GA₃ (150 mg.l⁻¹).

The plants were sprayed until they were run – off according to their treatments during evening hours, three spraying times were performed with 15 day intervals, first spraying was 60 days after seed sowing.

The obtained results were analyzed statistically, the means of single effects of plant growth regulators compared by t-test according to “Levene’s Test for Equality of Variances”, the

means of combined effects of plant growth regulators compared by Duncan’s Multiple Range Test at 5% probability level (Al-Rawi and Khalaf-Allah,1980). The statistical analysis was carried out using SPSS (Statistical Package for Social Sciences) program (Casanova *et al.*, 2004).

2.4 Determination of chemical composition

2.4.1 Moisture content (%):

Moisture content in the stems was determined by moisture meter L606 Wagner (Rev, 2004).

2.4.2 Chlorophyll content (%):

The total content of chlorophyll was measured by SPAD-502 chlorophyll meter, for each data three fully expanded leaves were used before harvesting (Shekhany, 2014).

2.4.3 Protein content (%):

The amount of protein was calculated by multiplying the value of nitrogen by 6.25. Micro kjeldahl was used to determine the nitrogen content of the leaves and calyxes when 300 mg of dried oven sample powder was digested with 5 ml sulfuric acid (98%) and 5 ml hydrogen peroxide (36%) and sodium hydroxide (40%) and boric acid distillation (Guebel *et al.*, 1991).

2.4.4 Total soluble solids (TSS%):

Total soluble solids of leaves and calyxes was measured by using hand refractometer (Atago 8469) as described by (Kim *et al.*, 2003).

2.4.5 Total flavonoids (mg.g⁻¹):

The maximum amount of flavonoids in the leaves and calyxes was determined by colorimetric aluminum chloride, using spectrophotometer at the absorbancy of 510 nm, the calculation of flavonoid amounts was computed from calibration curve as total flavonoid equivalent (mg)/ dry weight (g) (Kim *et al.*, 2003).

2.4.6 Total anthocyanin content (TAC) (mg.kg⁻¹):

TAC has been calculated by pH-differential anthocyanin pigments undergoing reversible

structural transformations with a change in pH evidenced by markedly different absorption spectra, this method was described by (Sutharut and Sudarat, 2012).

2.4.7 Ascorbic acid (mg.kg⁻¹):

The amount of ascorbic acid in the leaves and calyxes was calculated by the 2,6 dichlorophenol indophenols method as explained (Shintani, 2013).

2.4.8 Genistein, Hesperetin and Myricetin (mg.kg⁻¹):

The active substances (Genistein, Hesperetin and Myricetin) were extracted using the described method by (Obouayeba *et al.*, 2014) and measured the content of the oxidative leaves of the antioxidants through the duration of their retention by HPLC (High-Performance Liquid Chromatography) system by using column C18-ODS (25 cm×4.6 mm).

All chemical analysis was done in laboratory of department of the environment and water, Ministry of Science and Technology, Baghdad-Iraq.

3. RESULTS AND DISCUSSION

3.1 Effect of IAA

The results of table (3) shows that spraying of 100 mg.l⁻¹ IAA caused significant difference only in total flavonoids in the leaves of *H. sabdariffa* L. and the highest value was (43.25 mg.g⁻¹). Whereas, no significant differences obtained on moisture contents in the stem and other chemical contents in the leaves and calyxes. There is an agreement in result between the current experiment with those obtained by (Cui *et al.*, 2010) they found significant increases in total flavonoids of adventitious *Hypericum perforatum* roots by 0.5 and 1.0 mg.l⁻¹ IAA exogenous supplies. Flavonoids are also reasonable candidates for endogenous auxin transport regulators (Jacobs and Rubery, 1988).

Table (3) Effect of IAA on studied components of *H. sabdariffa* L.

Chemical contents	IAA mg.l ⁻¹						p-Values		
	0			100			Stem	leaves	calyxes
	stem	leaves	calyxes	Stem	leaves	calyxes			
Moisture content (%)	17.43	-	-	18.79	-	-	0.312	-	-
Chlorophyll (Spad) (%)	-	56.06	-	-	60.97	-	-	0.361	-
Protein (%)	-	28.19	17.48	-	23.24	20.78	-	0.190	0.038
TSS (%)	-	13.35	14.29	-	14.52	15.26	-	0.101	0.053
Total flavonoids (mg.g ⁻¹)	-	33.74	18.01	-	43.25	20.04	-	0.034	0.114
Anthocyanin (mg.kg ⁻¹)	-	103.55	716.65	-	111.59	782.24	-	0.125	0.104
Ascorbic acid (mg.kg ⁻¹)	-	80.38	15.22	-	89.64	16.74	-	0.100	0.050
Genistein (mg.kg ⁻¹)	-	16.98	29.08	-	19.82	31.32	-	0.052	0.068
Hesperetin (mg.kg ⁻¹)	-	2.35	4.03	-	3.31	5.22	-	0.060	0.055
Myricetin (mg.kg ⁻¹)	-	10.35	11.23	-	11.39	13.05	-	0.068	0.054

* Means a statistically significant difference of P<0.05 according to “Levene’s Test for Equality of Variances”

3.2 Effect of BA

Table (4) presents the effect of BA on water content in the stem and chemical contents in the leaves and calyxes of *Hibiscus sabdariffa* L. it can be seen that BA had significant effects on water content in the stem and all chemical components in the leaves and calyxes except (chlorophyll (%) and protein (%) contents) in the leaves. The highest values (19.53%, 15.21%, 45.77 mg.g⁻¹, 117.02 mg.kg⁻¹, 93.28 mg.kg⁻¹, 20.81 mg.kg⁻¹, 3.61 mg.kg⁻¹ and 11.87 mg.kg⁻¹) were obtained for (moisture content (%) in the stem and TSS (%) total flavonoids mg.kg⁻¹, anthocyanins mg.kg⁻¹, ascorbic acid mg.kg⁻¹, genitein, hesperetin and myricetin mg.kg⁻¹) respectively, but for calyxes the highest values were (21.71 %15.59%, 21.22 mg.kg⁻¹, 819.24 mg.kg⁻¹, 17.63 mg.kg⁻¹, 32.30 mg.kg⁻¹, 5.69 mg.kg⁻¹ and 13.81 mg.kg⁻¹) for (protein(%) TSS (%), total flavonoids mg.g⁻¹, anthocyanins mg.kg⁻¹, ascorbic acid mg.kg⁻¹, genitein mg.kg⁻¹, hesperetin mg.kg⁻¹ and myricetin mg.kg⁻¹) respectively, when 150 mg.l⁻¹ BA applied. Similar results recorded by Aycock *et al.* (1999) reported that the yield of cotton plants treated with cytokinin has increased significantly, and with Abdel Latef *et al.*, (2009) when they discovered that BA treatment showed noticeable stimulation of the soluble and total carbohydrate content of two Roselle cultivars tested. Moreover, Ramtin *et al.* (2015) revealed that the most water content is gained by spraying Carnation (*Dianthus caryophyllus* L.) plants by BA 50 µM (8.973%)

which was about two folds more than control (4.426%).

Table (4) Effect of BA on studied chemical components of *H. sabdariffa* L.

Chemical contents	BA mg.l ⁻¹						p-Values		
	0			150			Stem	leaves	calyxes
stem	leaves	calyxes	Stem	leaves	calyxes	Stem			
Moisture content (%)	16.7	-	-	19.53	-	-	0.030	-	-
Chlorophyll (Spad) (%)	-	59.96	-	-	57.07	-	-	0.597	-
Protein (%)	-	27.52	16.55	-	23.91	21.71	-	0.342	0.000
TSS (%)	-	12.66	13.97	-	15.21	15.59	-	0.000	0.000
Total	-	31.22	16.83	-	45.77	21.22	-	0.001	0.000
flavonoids (mg.g⁻¹)									
Anthocyanin (mg.kg ⁻¹)	-	98.12	679.6	-	117.0	819.2	-	0.000	0.000
Ascorbic acid (mg.kg ⁻¹)	-	76.73	14.33	-	93.28	17.63	-	0.000	0.000
Genistein (mg.kg ⁻¹)	-	15.99	28.09	-	20.81	32.30	-	0.000	0.000
Hesperetin (mg.kg ⁻¹)	-	2.04	3.56	-	3.61	5.69	-	0.001	0.000
Myricetin (mg.kg ⁻¹)	-	9.87	10.47	-	11.87	13.81	-	0.000	0.000

* Means a statistically significant difference of $P<0.05$ according to “Levene’s Test for Equality of Variances”

3.3 Effect of GA₃ on water content in the stem and chemical contents in the leaves and calyxes of (*H. sabdariffa* L.)

Table (5) shows that the maximum significant amount of moisture content (19.83%) has been registered with 100 mg.l⁻¹ GA₃. However, GA₃ treatment caused significant highest values of protein (%), TSS (%), total flavonoids mg.g⁻¹, anthocyanin mg.kg⁻¹, ascorbic acid mg.kg⁻¹, genistein mg.kg⁻¹, hesperetin mg.kg⁻¹ and myricetin mg.kg⁻¹ in the leaves and calyxes, except protein content in the leaves. Analogous results observed by (Hayssam *et al.*, 2011) they discovered that *H. sabdariffa* L. under non-saline condition, application of GA₃ enhanced growth characteristics (relative water content, anthocyanin and photosynthetic pigments (chlorophyll a, b and total chlorophyll). They showed that alleviating effects of GA₃ might be due to its role in the enhancement of carbonic anhydrase CA activity, the enzyme that catalyzes the hydration reversible CO₂ to HCO³⁻.

Table (5) Effect of GA₃ on studied chemical components of *H. sabdariffa* L.

Chemical contents	GA ₃ mg.l ⁻¹						p-Values		
	0			150			Stem	leaves	calyxes
stem	leaves	calyxes	Stem	leaves	calyxes	Stem			
Moisture content (%)	16.4	-	-	19.83	-	-	0.008	-	-
Chlorophyll (Spad) (%)	-	51.12	-	-	65.90	-	-	0.003	-
Protein (%)	-	27.77	16.98	-	23.66	21.29	-	0.280	0.005
TSS (%)	-	13.05	14.10	-	14.82	15.46	-	0.010	0.004
Total	-	32.39	17.21	-	44.59	20.84	-	0.005	0.002
flavonoids (mg.g⁻¹)									
Anthocyanin (mg.kg ⁻¹)	-	100.9	693.2	-	114.1	805.6	-	0.008	0.003
Ascorbic acid (mg.kg ⁻¹)	-	78.95	14.74	-	91.07	17.22	-	0.008	0.004
Genistein (mg.kg ⁻¹)	-	16.48	28.47	-	20.32	31.93	-	0.006	0.003
Hesperetin (mg.kg ⁻¹)	-	2.16	3.75	-	3.49	5.50	-	0.008	0.003
Myricetin (mg.kg ⁻¹)	-	10.12	10.89	-	11.62	13.39	-	0.005	0.006

* Means a statistically significant difference of $P<0.05$ according to “Levene’s Test for Equality of Variances”

3.4 Interaction effects of IAA, BA and GA₃

The impact of IAA, BA and GA₃ on water content in the stem and chemical contents in *H. sabdariffa* L. leaves and calyxes is displayed in table (6). It can be seen that there were significant differences in all chemical content parameters in the plant parts with spraying the three different plant growth regulators. The highest values of moisture content in the stem and chlorophyll (spad), TSS, total flavonoids, anthocyanin, ascorbic acid (vitamin C), genistein, hesperetin and myrecetin in the leaves were (24.97%, 77.13%, 16.20%, 59.67 mg.g⁻¹, 123.57 mg.kg⁻¹, 103.38 mg.kg⁻¹, 25.91 mg.kg⁻¹, 5.61 mg.kg⁻¹ and 12.90 mg.kg⁻¹ respectively) when sprayed with 100 mg.l⁻¹ IAA+150 mg.l⁻¹ BA+150 mg.l⁻¹ GA₃, by the way the highest value for protein (32.34%) was obtained in the leaves with the treatment 0 mg.l⁻¹ IAA+150 mg.l⁻¹ BA+150 mg.l⁻¹ GA₃, also, this treatment gave the highest values in the calyxes for protein, TSS, total flavonoids, anthocyanin, ascorbic acid (vitamin C), genistein, hesperetin and myrecetin (25.10%, 16.31%, 24.73 mg.g⁻¹, 896.41 mg.kg⁻¹, 19.36 mg.kg⁻¹, 35.71 mg.kg⁻¹, 7.05 and 15.89 mg.kg⁻¹ respectively). As we noticed, chemical contents of calyxes were superior in comparison with leaves. The rise in phenol content can be due to the increase in carbohydrate synthesis by application BA and or GA₃ (Sadak, 2005). Similar results have been reported by Hassanein *et al.* (2005) showing that

by applying the similar concentration of GA₃ and/or BA on Roselle calyxes, the anthocyanin content would increase significantly when the maximal value was reported at 100 mg.l⁻¹ of both GA₃ + BA, this result was referred to as an increase in the activity of phenylalanine ammonia lyase and tyrosine ammonia lyase in Roselle's shoot. Also, it is in agreement with Mukhtar (2008) when he found that total chlorophyll (%), vitamin C mg.kg⁻¹ and protein (%) content were increased with 100 mg.l⁻¹ IAA, 100 mg.l⁻¹ with 15% coconut milk. Moreover, our results are partially similar with those found by Hayssam *et al.* (2011) on chlorophyll *a*, *b*, total chlorophyll and anthocyanin the best results were obtained with GA₃ as compared to control. In comparison of our study other researches were obtained similar results, (Khandaker *et al.* 2018) best result of chlorophyll (spad) content obtained with 60 mg.l⁻¹ GA₃, and highest TSS content (2.47% Brix) was in the 90 mg.l⁻¹ IAA treatment.

Table (6) Effect of IAA, BA and GA₃ on studied chemical components of *H. sabdariffa* L.

Treatments	Chemical contents																			
	Chlorophyll (spad) (%)		Moisture content (%)		Protein (%)		TSS (%)		Total Flavonoids (mg.g ⁻¹)		Anthocyanin in (mg.kg ⁻¹)		Ascorbic acid (Vitamin C) (mg.kg ⁻¹)		Gestein (mg.kg ⁻¹)		Hesperetin (mg.kg ⁻¹)		Myricetin (mg.kg ⁻¹)	
	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes	Leaves	Calyxes
0	0	0	64.9	15.6	24.2	14.1	11.3	12.3	25.0	14.4	85.8	600.	68.9	12.3	13.5	25.7	1.26	21.1	8.60	8.59
			0 c	7 d	0 f	7 h	8 h	3 h	9 h	8 h	3 h	42 h	6 h	6 h	9 h	0 h	h	7 h	h	h
150			52.4	19.8	28.0	15.7	12.3	14.2	30.6	17.5	100.	702.	76.2	14.9	16.5	28.4	2.18	3.79	10.1	10.6
			3 f	7 b	0 d	8 f	9 f	8 f	7 f	9 f	16 f	38 f	1 f	3 f	9 f	2 f	f	f	3 f	1 f
0	150		36.7	15.2	28.2	16.6	13.8	14.6	31.5	18.6	108.	723.	79.2	15.3	17.4	29.3	2.34	4.08	10.5	11.4
			3 h	7 d	5 d	2 e	1 e	4 e	3 e	45 e	50 e	3 e	5 e	0 e	8 e	f	e	f	e	9 e
150			70.1	18.9	32.3	23.3	15.8	15.9	47.6	21.3	119.	840.	97.1	18.2	20.3	32.8	3.60	6.07	12.0	14.2
			7 b	3 c	4 a	6 b	1 b	5 b	9 b	6 b	77 b	31 b	0 b	3 b	5 b	0 b	c	b	9 b	4 b
100	0	0	58.6	15.7	27.1	15.3	11.9	13.9	28.7	15.5	93.2	632.	74.2	13.6	15.3	27.4	2.14	3.18	9.38	9.89
			0 e	3 d	0 e	4 g	9 g	7 g	7 g	8 g	5 g	18 g	0 g	6 g	6 g	9 g	g	g	g	g
150			63.9	15.5	30.8	20.9	14.8	15.2	40.3	19.6	113.	783.	87.5	16.3	18.4	30.7	2.59	5.09	11.3	12.8
			0 d	3 d	1 c	2 d	7 d	9 d	6 d	8 d	25 d	63 d	7 d	6 d	2 d	8 d	e	d	8 d	1 d
0	150		44.2	18.9	31.5	21.7	15.0	15.4	44.1	20.1	116.	816.	93.4	17.5	19.5	31.3	2.89	5.56	11.8	13.6
			3 g	3 e	4 b	8 e	3 e	7 e	9 e	6 e	28 e	74 e	2 e	9 e	8 e	1 e	d	e	9 e	1 e
150			77.1	24.9	3.50	25.1	16.2	16.3	59.6	24.7	123.	896.	103.	19.3	25.9	35.7	5.61	7.05	12.9	15.8
			3 a	7 a	g	0 a	0 a	1 a	7 a	3 a	57 a	41 a	38 a	6 a	1 a	1 a	a	a	0 a	9 a

* Values within each column followed with the same letters are not significantly different from each other according to Duncan's Multiple Range Test at the (0.05) level.

4. CONCLUSIONS

It is concluded that the application of 100, 150 and 150 mg.l⁻¹ of IAA followed by BA and GA₃ individually or in combination increased the studied phytochemical contents in leaves and calyxes of Roselle plant.

References

ABDEL LATEF, A. A., SHADDAD, M. A. K., ISMAIL, A. M. & AHMAD, M. F. A. 2009. Benzyladenine can alleviate saline injury of two roselle (*Hibiscus*

sabdariffa) cultivars via equilibration of cytosolutes including anthocyanins. *Int. J. Agric. Biol.*, 11, 151-157.

AHMED, Y.M., E.A. SHALABY & N.T. SHANAN, 2011. The use of organic and inorganic cultures in improving vegetative growth, yield characters and antioxidant activity of Roselle plants (*Hibiscus sabdariffa* L.). *Afr. J. Biotech.*, 10, 1988-1996.

ALI, H.M., SIDDIQUI, M.H., BASALAH, M.O., AL-WHAIBI, M.H., SAKRAN, A.M. & AL-AMRI, A., 2012. Effects of gibberellic acid on growth and photosynthetic pigments of *Hibiscus sabdariffa* L. under salt stress. *African Journal of Biotechnology*, 11, 800-804.

AL-RAWI, K.M. & KHALAF-ALLA, A. 1980. *Agriculture Experimental Design and Analysis*. Dar Al-Kutub for printing and publishing. Mosul - Iraq. (In Arabic).

ANTONY E, CHOWDHURY S.R. & KAR, G. 2003. Variations in heat and radiation use efficiency of green gram as influenced by sowing dates and chemical sprays. *Journal of Agrometeorology*, 5, 58-61.

AYCOCK, B., DUGGER, P. & RICHTER, D. 1999. Super start, Sul-15, GS-48 and GS-70 plant growth regulators carried with foliar fertilizers for cotton production. Proceedings of the Belt-Wide Cotton Conference, (BWCC'99), Orlando, Florida, USA, Jan, 1,71-73.

BIDWELL, R. G. S. 1979. *Plant physiology*. Second Edition. MacMillan Publishing Co, Inc, New York.

CASANOVA, E., VALDES, A.E., FERNANDEZ, B., MOYSET, L. & TRILLAS, M.I. 2004. Levels and immunolocalization of endogenous cytokinins in thidiazuron-induced shoot organogenesis in carnation. *Journal of Plant Physiology*, 161, 95-104.

CASTRO, N.E.A, PINTO, J.E, CARDOSO, M.G, MORAIS, A.R, BERTOLUCCI, S.K.V, SILVA, F.G. & DELU FILHO, N. 2004. Planting time for maximization of yield of vinegar plant calyx (*Hibiscus sabdariffa* L.). *Ciênc agrotec*, 28, 542-551.

CUI, X.H., CHAKRABARTY, D., LEE, E.J. & PAEK, K.Y. 2010. Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L. in a bioreactor. *Bioresour. Technology*, 101, 4708-4716.

ESTEFAN, G., SOMMER, R. & RYAN, J. 2013. *Methods of soil, plant, and water analysis: A manual for the west, Asia and North Africa region*. 3rd ed. ICARDA, Beirut, Lebanon.

FASOYRO, S.B., ASHAYE, O.A., ADEOLA, A. & SAMUEL, F.O. 2005. Chemical and storability of fruit flavoured (*Hibiscus*

- sabdariffa*) drinks. *World J. Agric. Sci*, 1, 165-168.
- FRIMIL, J. 2003. *Auxin transport—shaping the plant*. *Current Opinion in Plant Biol*, 6, 7–12.
- GEBREMEDIN, B., 2015. Influence of Variety and Plant Spacing on Yield and Yield Attributes of Roselle (*Hibiscus sabdariffa* L.). *Science, Technology, and Arts Research Journal*, 4, 25-30.
- GUEBEL, D.V., NUDEL, B.C. & GIULIETTI, A.M. 1991. A simple and rapid micro-Kjeldahl method for total nitrogen analysis. *Biotechnology Techniques*, 5, 427–430.
- HASSANEIN, R.A., HEMMAT, K.I., KHATTAB, H.K.I. & SADAK, M.S. 2005. Increasing the Active Constituents of Sepals of Roselle (*Hibiscus Sabdariffa* L.) Plant by Applying Gibberellic Acid and Benzyladenine. *J. Appl. Sci. Res*, 1, 137-146.
- JACOBS, M., & RUBERY, P. H. 1988. Naturally occurring auxin transport regulators. *Science*, 241, 346-349.
- KADIRI, M., MUKHTAR, F. & AGBOOLA, D.A. 1997. Responses of some Nigerian vegetables to plant growth regulator treatments. *Rev. Biol. Trop*, 44 / 45, 23-28.
- KHANDAKER, M. M., AZAM, H. M., ROSNAH, J., TAHIR, D. & NASHRIYAH, M. 2018. The effects of application of exogenous IAA and GA3 on the physiological activities and quality of *Abelmoschus esculentus* (Okra) var. Singa 979. *Pertanika Journal of Tropical Agricultural Science*, 41, 209-224.
- KIM, D.O., JEONG, S. W. & LEE, C. Y. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem*, 81, 321-326.
- MOSTAFA, G. G. & ABOU ALHAMD, M. F. 2011. Effect of Gibberellic Acid and Indole 3-acetic Acid on Improving Growth and Accumulation of Phytochemical Composition in *Balanites aegyptiaca* Plants. *American Journal of Plant Physiology*, 6, 36-43.
- MUKHTAR, F.B. 2008. Effect of Some Plant Growth Regulators on the Growth and Nutritional Value of *Hibiscus sabdariffa* L. (Red sorrel). *Int. Jor. P. App. Scs*, 2, 70–75.
- OBOUAYEBA, A.P., DJYH, B. N., SEKOU, D. & JOSEPH, D. 2014. Phytochemical and antioxidant activity of Roselle (*Hibiscus Sabdariffa* L.) petal extracts. *Journal of Pharmaceutical, Biological and Chemical Sciences*, 5, 1453-1465.
- OSMAN, M., FARUQ, G., SABERI, S., ABDUL MAJID, N., NAGOOR, N. H. & ZULQARNAIN, M. 2011. Morpho-agronomic analysis of three roselle (*Hibiscus sabdariffa* L.) mutants in tropical Malaysia. *Australian Journal of Crop Science*, 5, 1150-1156
- PULLAIAH, T., SUBBA RAO, M.V. & SREEDEVI, E. 2017. *Plant Tissue Culture: Theory & Practicals*, 2nd Ed. Jodhpur: Scientific Publishers.
- RAMTIN, A., S. KALATEJARI, NADERI, R. & MATINIZADEH, M. 2015. Effect of Pre-Harvest Foliar Application of Benzyl Adenine and Salicylic Acid on Carnation Cv. Spray and Standard. *Biological Forum – An Int. J.*, 7, 955-958.
- REV, B. 2004. Wepi wagner part. Wagner Electronicproducts, Inc. #500-60601-002. <http://www.wagnermeters.com>
- RIAZ, G. & CHOPRA, K. 2018. A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. *Biomedicine and Pharmacotherapy*, 102, 575-586.
- SADAK, M.S., 2005. *Physiological studies on the interaction effects of gibberellic acid and benzyladenine on Roselle (Hibiscus sabdariffa L.) plant*. Ph.D. Thesis, Faculty of Science, Ain Shams University, Egypt.
- SHEKHANY, H.K.A. 2014. *Influence of magnetized water on the ability of nutrient uptake and the growth of two cultivars of Pistachio (Pistacia vera L.) seedlings*. M.Sc. thesis, University of Salahaddin.
- SHINTANI, H., 2013. HPLC Analysis of Ascorbic Acid (Vitamin C). *Pharmaceutica Analytica Acta*, 4, 1000234.
- SUTHARUT, J. & SUDARAT J. 2012. Total Anthocyanin Content and Antioxidant Activity of Germinated Colored Rice. *International Food Research Journal*, 19, 215-221.