SVU-International Journal of Agricultural Science
Volume 2 Issue (2) pp.: 476-483, 2020
Print ISSN 2636-3801 | Online ISSN 2636-381X
Doi: 10.21608/svuijas.2020.52563.1061

RESEARCH ARTICLE

Effect of salicylic acid, moringa leaves extract and seaweed extract on growth, yield and quality of roselle, *Hibiscus sabdariffa* L., under Aswan conditions

Zakria Y.,[1] S.S.A. Abdel-Rahman[2], W.S. Soliman[1,*], S. Salahelidn[1]
[1]*Horticulture Department, Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt..*
[2]*Horticulture Department, Faculty of Agriculture, Assiut University, Assiut, Egypt*

Abstract

Bio-stimulants play a vital role in the sustainable development of horticultural crops. The aim of this study was to assess the effect of synthesis stimulator (salicylic acid) and bio-stimulants (moringa extract, seaweed extract) on growth, yield and quality of Roselle, *Hibiscus sabdariffa* L., plants. The recorded growth parameter included plant height, number of lateral branches per plant, branch diameter, and leaf area. For yield traits, the calyxes' fresh and dry weight was recorded. Leaf pigments included chlorophyll a, b, and carotenoids. Anthocyanin content in calyxes was measured. The experiment was conducted during 2018 and 2019 seasons, and was designed in completely randomized system. The results showed that application of bio-stimulants showed positive effects on all traits, and the best results were shown under treatment of seaweed, followed by moringa leaves extract. There were positively associations of calyxes yield and its anthocyanin content with other growth traits and photosynthetic pigments. These results highlighted the important role of bio-stimulants, especially seaweed extract, as a bioactive stimulant. Therefore, it is recommended to include seaweed extract in fertilization programs for achieving sustainable production system of such crops.

Keywords: Anthocyanin; Moringa extract; Roselle plant; Salicylic acid; Seaweed extracts**.**

Introduction

Natural and synthetic growth stimulators play an important role in promoting growth and development of crops. For decades, such plant growth stimulators have been used extensively in crop production, whether by soil application or foliar spraying. Plant stimulators have positive effects not only on plant growth and nutrition, but also on abiotic and biotic stress tolerance (du Jardin, 2015). For example, salicylic acid as a synthetic stimulator regulates several functions in plant including plant resistance to environmental stresses such as drought and salinity (Arfan et al., 2007) and chilling (Farooq et al., 2008). In addition, natural plant bio-stimulants are important in sustainable horticulture production systems. There are several studies that have reviewed the role of bio-stimulants in relation to promoting growth and nutrient availability (Colla and Rouphael, 2015; du Jardin, 2015). In addition to numerous studies

***Corresponding author:** W.S. Soliman
Email: wagdi79@agr.aswu.edu.eg
Received: December 6, 2020
Accepted: December 19, 2020
Published: December 26, 2020

that have reviewed broadly defined categories of bio-stimulants such as protein hydrolysates, seaweed extracts, silicon, chitosan, humic acid, and fulvic acid (Van Oosten et al., 2017). The results showed the positive effects of seaweed on plant productivity and quality (Layek et al., 2017; Pramanick et al., 2017; Mzibra et al., 2018). This is in addition to the beneficial effects of seaweed extracts, including betaines, polysaccharides, amino acids, polyamines, and vitamins (Lötze and Hoffman, 2015; Singh et al., 2015). Other studies demonstrated the impact of seaweed extracts on plant stress tolerance, including biotic and abiotic stresses (Mansori et al., 2014; Arioli et al., 2015). On the other hand, moringa leaf extracts positively improve seeds germination, plant growth and yield, nutrient use efficiency, crop quality characters, as well as tolerance to environmental stresses (Abdel-Rahman and Abdel-Kader, 2020; Zulfiqar et al., 2020).

Roselle (*Hibiscus sabdariffa* L.) plant is a botanical species belonging to the Malvaceae family. It is probably native to a tropical central and west of Africa, and it is recognized as tropical and sub-tropical shrub (Dhar et al., 2015). Roselle plays an important role in income and subsistence providing for rural farmers in developing countries. The part of the plant used for nutritional and medicinal purposes is the calyxes, which have nutritional values and medicinal benefits. Roselle calyxes are rich in nutrients such as anthocyanins, organic acids, pectin, phenolic compounds and vitamins. It is commonly used for reducing chronic diseases (Wu et al., 2018), as well as acts as an antimicrobial (Khalaphallah and Soliman, 2014).

Natural and synthetic growth stimulators play an important role in the sustainable management of horticultural crops. The aim of this experiment was to examine the effects of salicylic acid, moringa leaves extract as well as seaweed extract on the vegetative growth, yield and quality of Roselle, *Hibiscus subdariffa* L., plants. The hypothesis of this study is that bio-stimulants are effective alternatives to mineral fertilizers especially under organic farming system.

Material and methods

The present study was carried out during the two successive seasons of 2018 and 2019 at Agricultural Experimental Farm, Faculty of Agriculture and Natural Resources, Aswan University, Sahary city, Aswan, Egypt. Seeds of Roselle (Sobahia dark variety) were obtained from the Agricultural Research Centre, Giza, Egypt, and were directly sown in the field on 15th of April of each year. The experiment was designed in a completely randomized complete design with four replicates. Four treatments were used; moringa leaves extract (5%), Seaweed extract (0.3%), Salicylic acid (400 ppm) as well as control. Each experimental unit (plot) included two rows, 50 cm apart and 3 m length. The seeds were sown in hills, 30 cm apart, with a total of 20 plants in each plot. Drip irrigation system was used for irrigation purpose and normal agricultural practices were carried out. The treatments were foliar spraying at three times; at 45, 75, and 105 days from planting. The plants were harvested during October of each year, and the data has been collected.

1. Vegetative growth and yield traits

The vegetative growth parameters were recorded including plant height (cm), number of lateral branches per plant disturbed on the main branch, branch diameter (cm), and leaf area (cm^2). For yield traits, the calyxes were separated and fresh weight per plant were recorded, then calyxes were air dried in shade conditions for one week, and dry weight per plant were recorded. The total yield of fresh and dry calyxes was calculated per Fadden (one Fadden equals to 4200 m^2).

2. Photosynthetic pigments:

Samples of fresh leaves were randomly collected from the mid-part of plants of each treatment in the early morning. Leaf pigments were measured by extraction of 50 g fresh leaves by 10 mL acetone 80%. The developed color was colorimetrically determined using SPECTROstar Nano (BMG LAbTECH GmbH, Germany) at wave length of 663, 644 and 452.5 nm according to Metzner et al. (1965). Chlorophyll "a", "b", and carotenoids contents were calculated taking into consideration the dilution factor. The concentration of pigment fractions was determined as mg/ml using the following equations:

Chlorophyll "a" =10.3E663 – 0.918E644

Chlorophyll "b" =19.7E644 – 3.87E663

Carotenoids =4.2E452.5 – (0.0264 Chl.a + 0.426 Chl.b)

Finally, the pigment fractions were calculated as mg/g fresh weight of leaves.

3. Determination of total anthocyanin

Anthocyanin content in calyxes was determined according to Lee et al. (2005). This method is based on the monomeric anthocyanin pigments reversibly changing color with a change in pH; the colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance at 520 nm at those two pH is proportional to the pigment concentration.

Known weight (200 mg) of air-dried calyxes was extracted using extraction solution (15 ml methanol 75% acidic with hydrochloric acid 1%) and incubated overnight at 4°C in the dark. The buffer solution (sodium acetate, pH 4.5) was added to 1 ml extraction, and the buffer solution (potassium chloride, pH 1.0) was added to another 1 ml extraction. The absorption of anthocyanin was colorimetrically measured at both pH at 520 and 700 nm using SPECTROstar Nano (BMG LAbTECH GmbH, Germany). The absorbance was measured within 20–50 min of preparation, and acidic methanol was used as blank.

The concentration of anthocyanin was calculated in the samples and expressed as cyanidin-3-glucoside (Cyd-3-Glu) equivalents (mg/100 g), as follows:

$$\frac{A \times MW \times DF \times 1000}{\in \times 1}$$

where A = (A520 nm − A700 nm) pH 1.0 − (A520 nm − A700 nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for Cyd-3-Glu; DF = dilution factor = 1.5; l = path length in cm; ∈ = 26,900 molar extinction coefficient of Cyd-3-Glu.

4. Statistical analysis:

Data were subjected to statistical analysis using one-way ANOVA. The least significant differences (L.S.D.) was used to analyze significance among the mean values of these parameters (Snedecor and Cochran, 1989). All statistical analyses were carried out using JMP (ver 4. SAS Institute, Cary, NC, USA).

Results and discussion

It was clear that spraying the Roselle plants with growth stimulators significantly increased vegetative growth parameter; plant height, number of branches, branch diameter and leaf area compared to untreated plants (Table 1).

Seaweed extract gave the tallest plants (153.8 and 141.5 cm), highest number of branches (almost 10 branches per plant), widest branch diameter (1.60 and 1.55 cm) and biggest leaf area (29.10 and 29.45 cm2) for the first and second seasons, respectively. The highest calyxes yield was obtained also with plants treated with seaweed extract (Table 2) as follow; calyxes fresh weight per plant (37.63 and 34.50 g), calyxes dry weight per plant (13.45 and 12.50 g), calyxes fresh weight per Fadden (668.9 and 613.3 kg), and calyxes dry weight per Fadden (239.1 and 222.2 kg) for the first and second seasons, respectively. In the same line, vegetative growth and yield associated with significantly increased in plant pigments content (Table 3). Plants treated with seaweed extract showed the highest content of chlorophyll a (2.33 and 2.28 mg/g), chlorophyll b (0.82 and 0.92 mg/g), carotenoids (0.61 and 0.65 mg/g) and total chlorophyll (3.46 and 3.24 mg/g) for the first and second seasons, respectively. In addition, it was clear that spraying plants with growth stimulators significantly increased quality of Roselle plants expressed by anthocyanin content in calyxes (Table 4). Seaweed extract gave the highest values of anthocyanin content (7.96 and 8.41 mg/g) compared to the control for the first and second seasons, respectively.

Table (1): Effect of salicylic acid (SA), moringa leaves extract, and seaweed extract on plant height (cm), branch number, branch diameter (cm), and leaf area (cm^2) in the 1st and 2nd seasons. Least significance difference (LSD) was calculated at probability of 0.05.

	Plant height		Branch number		Branch diameter		Leaf area	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	121.5^{a}	125.8^{a}	5.75^{a}	5.53^{a}	1.25^{a}	1.25^{a}	22.94^{a}	20.40^{a}
SA	134.3^{ab}	131.5^{b}	7.25^{b}	7.50^{b}	1.48^{ab}	1.33^{a}	25.71^{ab}	24.63^{b}
MLE	144.3^{bc}	137.0^{c}	8.50^{c}	8.75^{b}	1.58^{b}	1.50^{b}	28.43^{b}	27.33^{c}
Seaweed	153.0^{c}	141.5^{d}	9.50^{cd}	9.75^{c}	1.60^{b}	1.55^{b}	29.10^{b}	29.45^{cd}
LSD 0.05	18.3	4.3	1.22	1.48	0.28	0.10	3.99	2.27

Table (2): Effect of salicylic acid (SA), moringa leaves extract, and seaweed extract on calyxes fresh weight (FW) and dry weight (DW) per plant (g) as well as calyxes fresh weight (FW) and dry weight (DW) per Fadden (kg) in the 1st and 2nd seasons. Least significance difference (LSD) was calculated at probability of 0.05.

	Calyxes FW per plant		Calyxes DW per plant		Calyxed FW per Faddan		Calyxed DW per Faddan	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	29.23^{a}	27.50^{a}	8.75^{a}	8.25^{a}	519.6^{a}	488.9^{a}	155.6^{a}	146.7^{a}
SA	32.28^{b}	30.25^{ab}	10.75^{b}	10.75^{b}	573.8^{b}	537.8^{ab}	191.1^{b}	191.1^{b}
MLE	34.95^{c}	32.25^{bc}	12.75^{c}	11.88^{c}	621.3^{c}	573.3^{bc}	226.7^{c}	211.1^{c}
Seaweed	37.63^{d}	34.50^{c}	13.45^{cd}	12.50^{cd}	668.9^{d}	613.3^{c}	239.1^{cd}	222.2^{cd}
LSD 0.05	1.78	3.07	0.84	0.96	31.6	54.5	15.0	17.1

Natural and synthesis stimulators contribute to sustainable, high-output, and low-input crop productions. A bio-stimulant is defined as any substance or microorganism added to plants to improve nutrition efficiency, abiotic stress resistance and/or quality traits (du Jardin, 2015).

Table (3): Effect of salicylic acid (SA), moringa leaves extract, and seaweed extract on leaf pigments; chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll content (mg/g) in the 1st and 2nd seasons. Least significance difference (LSD) was calculated at probability of 0.05.

	Chlorophyll a		Chlorophyll b		Carotenoids		Total chlorophyll	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	1.09a	1.38a	0.48a	0.62a	0.36a	0.44a	1.58a	2.35a
SA	1.73b	1.83b	0.67b	0.77b	0.43b	0.55b	2.40b	2.60a
MLE	2.08c	1.96c	0.80c	0.80b	0.53c	0.62c	2.88c	2.75a
Seaweed	2.33d	2.28d	0.82cd	0.92c	0.61d	0.65cd	3.46d	3.24b
LSD 0.05	0.08	0.07	0.05	0.10	0.01	0.04	0.22	0.41

Table (4): Effect of salicylic acid (SA), moringa leaves extract, and seaweed extract on calyxes' anthocyanin content (mg/g) in the 1st and 2nd seasons. Least significant difference (LSD) was calculated at probability of 0.05.

	Anthocyanin	
	1st	2nd
Control	5.40a	4.04a
SA	5.83ab	5.07a
MLE	7.82ab	6.76ab
Seaweed	7.96b	8.41b
LSD 0.05	2.51	3.06

Kauffman et al. (2007) classified bio-stimulants into three major groups on the basis of their source as followed; (i) humic substances, (ii) hormone containing products, and (iii) amino acid containing products. On the other hand, previous studies have demonstrated that Moringa leaf extracts improve seed germination, plant growth, yield, nutrient utilization efficiency, quality characteristics of crops, as well as tolerance to abiotic stresses (Abdel-Rahman and Abdel-Kader, 2020; Zulfiqar et al., 2020). Moringa extracts are considered an suitable alternative source of inorganic fertilizers due to their high content of micro and macro mineral nutrients, protein and essential amino acids, which contribute to supplement the nutritional requirements of crops (Yasmeen et al., 2014). Latif and Mohamed (2016) attributed growth and yield responses when treated with Moringa to the presence of growth-promoting hormones in Moringa, as analyzes showed that Moringa extract contains antioxidants, gibberellins, IAA and ABA (Rady and Mohamed, 2015; Azam et al., 2020). The results of this study showed that seaweed extract had significantly positive effects on plant growth, yield and quality parameters. In this study, seaweed led to the highest vegetative growth, yield, pigment content and anthocyanin contents followed by moringa leaf extract. It was obvious that bio-stimulants (seaweed and moringa extracts) had better results compared to the synthesis growth stimulator (salicylic acid). Seaweed is classified as humic substance according to Kauffman et al. (2007) who explained the positive effects of seaweed in improving plant growth and quality to their containing specific amounts of active plant growth substances such as auxins, cytokinins, or their derivatives. Also, seaweed extracts include polysaccharides laminarin, alginates and carrageenans and their breakdown products as well as micro- and macronutrients (Khan et al., 2009; Craigie, 2011).

Conclusions

From the results of this study, it can be concluded that treatment with bio-stimulants, especially seaweed extract, led to significant increases in growth, yield and quality traits. These increases may due to increases in the metabolites and chlorophyll synthesis which contribute to raising the efficiency of the photosynthesis process, compounds biosynthesis and enzymes activity for growth resulting in improving the yield traits as well as quality traits through an increase of anthocyanin synthesis. Therefore, it can be recommended to use seaweed and moringa extracts, as an effective alternatives to regular fertilization, which contributes to the sustainable development of horticultural crops.

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