Evaluation of quality and growth of roselle
(*Hibiscus sabdariffa* L.) as affected by bio-fertilizers

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Evaluation of quality and growth of roselle (Hibiscus sabdariffa L.) as affected by bio-fertilizers

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ABSTRACT
Roselle (Hibiscus sabdariffa L.) plant is a valuable medicinal crop in arid and semi-arid regions. The use of microbes as bio-fertilizers in enhancing crop production is more favorable than chemical fertilizers due to food safety. A pot experiment was conducted to explore the effect of Azotobacter chroococcum and Azospirillum brasilense as a bio-fertilizer on the growth, yield, and quality of roselle plants. Roselle seeds were mixed with the tested bio-fertilizer and cultivated on plastic pots filled with a sandy clay loam soil. The bio-fertilization significantly increased the growth, nutrients uptake, yield, and quality of roselle plants compared to the untreated plants. The inoculation of roselle plants with the bio-fertilizer increased the total chlorophyll, carotenoid, total anthocyanin (TAC), and total flavonel (TF) by 16.45, 26.10, 8.44, and 14.27%, respectively, above the control. The bio-fertilization increased the soil available nitrogen by 14.33% above the control, and increased the uptake of N, P, and K by 18.8, 17.81, and 12.75%. The bio-fertilization not only increased the quality of roselle plants but also increased the fresh and dry weights of sepals yield by 5.89 and 3.55%, respectively.

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KEYWORDS
Azospirillum; Azotobacter; nitrogen; Roselle

Introduction
Roselle (Hibiscus sabdariffa L), family Malvaceae, is known commonly as Karkade. It is also known under other different names e.g., viz roselle, razelle, sorrel, red sorrel, Jamaica sorrel, Indian sorrel, Guinea sorrel, sour-sour, and queensland jelly plant (Moroton 1987). Medicinal plants are used throughout the world, and the regulations defining their proper use, such as identification of the correct species and page verification of the presence, purity, and concentration of the required chemical compounds, are widely recognized (Palhares et al. 2015). The global market of herbal medicines products is estimated at $83 billion US and continues to grow (WHO 2011). According to the WHO (2011), 65 to 80% of the populations of developing countries currently use medicinal plants as remedies. The trend of crops cultivated area in Egypt was increased during the period from 1990 to 2005 with growth rate reached to 2.5% and average cultivated area of 24.1 thousand hectare (Mohamed 2012). Also medicinal plants considered among the promising crops on which can increase Egypt income from foreign currency (Mohamed 2012). However, the value of its exports is estimated at about 6.6% of the total value of the Egyptian agricultural exports representing around 0.39% of the total crop-area (FAO 2018). Egypt has a lot
of ingredients that help medical and aromatic plants to be flourished, such as climate, manpower, proper soil, and availability of reclaimed land areas (Eissa 2014; Eissa, 2016a; El Naim et al. 2017; Eissa et al. 2018a).

Roselle is an important crop in tropical and sub-tropical regions (El Naim et al. 2017). The roselle plant can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt, and Mexico (El Naim et al. 2017). The main producers of roselle blossoms are Egypt, Sudan, Mexico, Thailand, and China (FAO 2018). The economical part of the plant is the fleshy calyx (sepals) surrounding the fruit (capsules) (El Naim et al. 2017). In Sudan, fully developed fleshy calyx is peeled off from the fruit by hand and dried naturally under shade to give the dry (calyx) (El Naim et al. 2017). Roselle is an annual plant; it is 0.5 to 1.5 m height and has a bushy shape with somewhat dense canopy of dark green leaves (El Naim et al. 2017). Roselle has many industrial and domestic uses; it is used as a beverage in the Sudan, where the dried calyx is soaked in water to prepare a colorful cold drink (El Naim et al. 2017). Traditionally the product has been used for medicinal purposes for relief of sour throat and for healing wounds as an anti-septic (El Naim et al. 2017). Anthocyanin pigments in roselle plant play a vital role in protection of the plant against biotic and abiotic stresses (Middleton, Kandaswami, and Harborne 1986). Anthocyanins are pigments from the flavonoid family of phenylpropanoid compounds that are responsible for the blue, purple, and red colors of leaves, flowers, and fruits (Middleton, Kandaswami, and Harborne 1986). As dietary components, they also have beneficial effects on human health, as they provide a source of antioxidants, reduce the incidence of coronary heart disease, and exhibit anticancer activity (Koes, Verweij, and Quattrocchio 2005).

Bio-fertilizers have been used in crop production for decades. The main functions of the microbes in bio-fertilizers are: (1) to supply nutrients to crops; (2) to stimulate plant growth, e.g., through the production of plant hormones; (3) to control or inhibit the activity of plant pathogens; and (4) to improve soil structure (Umesha, Singh, and Singh 2018). These increases in vegetative growth might be due to the increases in the soil microbial flora which happened by bio-fertilization (Zaki et al. 2012). Using of bio-fertilizers that contain different microbial strains has led to a decrease in the use of chemical fertilizers and has provided high quality products free of agrochemicals harmful and safe for human consumption (El Naim et al. 2017). Furthermore, the application of these bio-fertilizers also supports the conditions of root growth, increase the growth of the aboveground parts and finally improve the biological functions of the plant (El Azab and El Dewiny 2018).

Azotobacter chroococcum and Azospirillum brasilienise are non-symbiotic nitrogen fixers and they are well known as bio-fertilizers (Narula et al. 2005; Kahil, Hassan, and Ali 2017). The beneficial impact of N fixing bacteria may be due to the direct improved of plant growth promotion by the production of plant growth regulators (Umesha, Singh, and Singh 2018)). Inoculation with Azotobacter sp. increased the yield and nitrogen uptake by wheat and cotton (Narula et al. 2005). The application of bio-fertilizer increased the plant growth that increased hormones production such as GA and IAA hormones effects on plant growth (Hamidi et al. 2008). These growth promoters increase the vegetative growth (Hassan 2009).

Combined inoculation with Azotobacter chroococcum and Azospirillum brasiliense may result increases in plant growth, crop yield, and nutrients uptake (El Naim et al. 2017). There is little information available about the growth and quality of roselle plants as affected by the inoculation with a bio-fertilizer contains Azotobacter chroococcum and Azospirillum brasiliense. The current study aims to investigate the effect of bio-fertilization on the growth, yield, and quality of roselle.

Materials and methods

Pot experiment

Soil samples (0–30 cm) were collected from the Agricultural Experimental Farm of the Faculty of Agriculture, AL-Azhar University, Assiut, Egypt, which is located at 27° 12' 16.67" N latitude
and 31° 09' 36.86" E longitude in 2017. The collected soil samples were sieved to pass through a 2 mm stainless steel sieve. Plastic pots, 40 × 40 cm, were filled with 20 kg of the studied soil. The tested bio-fertilizer consists of a mixture of *Azotobacter chroococcum* and *Azospirillum brasilense* as non-symbiotic nitrogen fixers and contains 1 × 10⁸ colony mL⁻¹. The tested bio-fertilizer was obtained from the National Research Center, Giza, Egypt. The source of the roselle (*Hibiscus sabdariffa* L.cv. Sabhia 17) seeds was the Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Roselle seeds were washed with water, treated with a 10% Arabic gum as an adhesive, and mixed with the bio-fertilizer for 30 min then transplanted in the pots. Five seeds of rosella were sown in each pot; after emergence plants were thinned to two plants per pot. The plants were irrigated regularly to keep the soil moisture content near field capacity. Compost (3.47 g/kg), urea (46% N) at a rate of 0.20 g/kg, calcium super phosphate (15.5% P₂O₅), and 0.075 g/kg of potassium sulfate (48% K₂O) were added to the soil. The rate of mineral fertilization was added according to the Egyptian Ministry of Agriculture and Land Reclamation. The recommended fertilizers were added to the pots before the sowing and during the soil preparation. Plant samples were collected at full blooming and at harvest stage. Plant growth parameters i.e., plant height, number of branches per plant, number of sepals per plant, fresh and dry weight of plant were recorded. All the collected samples were cleaned, washed with tap and distilled water, air dried, and then dried in oven at 70°C until constant weight, ground, and stored for chemical analysis. Representative dry samples were taken from each replicate for chemical analysis.

**Analysis of physiochemical properties of the studied soil**

Table 1 shows some physiochemical properties of the soil used in the experiment. Particle-size distribution was carried out by using the pipette method according to (Burt 2004). The pH of soil was measured in 1:2.5 (soil: water) suspension and the electrical conductivity (EC) was measured in 1:2.5 extract (Burt 2004). Soil organic matter was determined by wet oxidation method by K₂Cr₂O₇ 1 N and H₂SO₄ (Burt 2004). Available soil nitrogen was extracted with 1% K₂SO₄ at a ratio of 1:5. The extract was distilled using a micro Kjeldahl’s distilling unit in the presence of 1 g Devarda’s alloy. After the distillation, available nitrogen (NH₄⁺ + NO₃⁻) content was determined in the distillate by titrating with standardized 0.01 N H₂SO₄ (Burt 2004). The available soil phosphorus was extracted from 5 g of soil sample using 100 mL of NaHCO₃ buffered at pH 8.5 according to Burt (2004). Phosphorus in the extract was determined by using the phosphomolybdic acid and stannous chloride method and measured by spectrophotometer at 660 nm (Burt 2004).

<table>
<thead>
<tr>
<th>Properties</th>
<th>0–30 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>53.5</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>22.3</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>24.2</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Field capacity (v%)</td>
<td>30</td>
</tr>
<tr>
<td>Witting point (v%)</td>
<td>21.6</td>
</tr>
<tr>
<td>CaCO₃ (%)</td>
<td>1.5</td>
</tr>
<tr>
<td>pH (1:2.5 suspension)</td>
<td>8.14</td>
</tr>
<tr>
<td>ECₑ (dS m⁻¹)</td>
<td>0.92</td>
</tr>
<tr>
<td>Organic matter (g kg⁻¹)</td>
<td>6.0</td>
</tr>
<tr>
<td>Total N (mg kg⁻¹) Total P (mg kg⁻¹) Total K (mg kg⁻¹)</td>
<td>300 300 400</td>
</tr>
<tr>
<td>Available N (mg kg⁻¹)</td>
<td>55</td>
</tr>
<tr>
<td>Available Olsen P (mg kg⁻¹)</td>
<td>8.5</td>
</tr>
<tr>
<td>Available K (mg kg⁻¹)</td>
<td>98.5</td>
</tr>
</tbody>
</table>

Each value represents a mean of three replicates.

ECₑ: the Electric Conductivity of the saturated soil extract.
2004). The available soil potassium was extracted from 5 g of soil sample using 50 mL by ammonium acetate 1 M at pH 7.0 and measured by flame photometer (Burt 2004). Total nitrogen in soil samples were digested using 20 mL of a mixture of 7: 3 ratio of sulfuric to perchloric acids. Digested samples were distilled with 20 mL of 40% sodium hydroxide using a micro Kjeldahl’s distilling unit. After the distillation, total nitrogen content was determined in the distillate by titrating with a standardized 0.01 N H₂SO₄ (Burt 2004). Total phosphorus was measured in the soil samples by digestion using a mixture of 7: 3 ratio of sulfuric to perchloric acids. Total calcium carbonate was determined by Collin’s calcimeter according to (Burt 2004). The values of field capacity and permanent wilting point were determined using the pressure cooker and pressure membrane apparatus. A saturated undisturbed and disturbed soil samples were equilibrated at suction pressures of 0.33 and 15 bar, respectively, according to (Burt 2004).

**Plant sample analysis**

Plant samples were taken and washed with deionized water, oven-dried at 70°C, mill ground, and kept for chemical analysis. Dried grounded plant material of 0.2 g was digested using 10 mL of a mixture of 7: 3 ratio of sulfuric: perchloric acids (Burt 2004). Total nitrogen was measured in the digested sample by distillation using a mixture of 20 mL of 40% sodium hydroxide using a micro Kjeldahl’s distilling unit (Burt 2004). Phosphorus was measured in the extract by using the chlorostannous, ammonium–molybdate method, while K was measured by flame photometer (Burt 2004).

The photosynthetic pigments were extracted from a definite fresh leaf sample in 5 mL of 95% ethyl alcohol in a test tube at 60°C, until colorless. Then the total volume completed into 10 mL with 95% ethyl alcohol and absorbance readings were determined with a spectrophotometer. Chlorophylls and carotenoids concentrations were calculated as mg/g fresh weight at 663, 644 and 452 nm using the following equations (Lichtenthaler 1987).

\[
\text{Chl.} a = (13.36 * A_{663}) - (5.19 * A_{644})
\]

\[
\text{Chl.} b = (27.49 * A_{644}) - (8.12 * A_{663})
\]

\[
\text{Carotene} = \{(1000 * A_{452}) - (2.13 * \text{Chl.} a) - (9.76 * \text{Chl.} b)\}/209.
\]

Total anthocyanin (TAC) and total flavonel (TF) were extracted from 1 g of dried sepals by adding 10 mL of (85:15) ethanol (96%): HCl 1.5 M. Then the sample was transferred to 50 mL beaker, covered and kept overnight in the refrigerator at a temperature of 4°C. Absorption of solution was measured by spectrophotometer at wavelength 535 nm for TAC and 374 nm for TF. The method was modified of Lee and Francis (1971).

\[
\text{TAC (mg/100 g dry weight)} = (A_{535} \times V \times 100)/(98.2 \times W).
\]

\[
\text{TF in mg/100g} = (A_{374} \times V \times 100)/(76.5 \times W).
\]

where \(V\) = total volume extract (mL); \(W\) = weight sample (g).

**Statistical analysis of data**

The trial was arranged in a complete randomized design (CRD) with eight replicates. To test the significance of difference between the studied plants, one-way-ANOVA and Duncan test were used. The statistical analysis was performed with SPSS statistical program.
Results and discussion

Effect of bio-fertilizer on the growth of roselle plants

Figure 1 shows some growth parameters of roselle plants at full blooming stage. The recorded growth characteristics include fresh and dry biomass, plant high, root length, and number of sepals and branches. The bio-fertilization with microorganisms significantly \((p < 0.05)\) increased the fresh, dry weights, plant high, and number of sepals and branch. The increases were 7.17, 4.33, 3.14, 32.16, and 10.23% above the control. The bio-fertilization increased the growth of roselle (Hassan 2009) and cruciferous vegetables (Zaki et al. 2012). These increases in vegetative growth may be due to increase in the soil microbial flora, which occurs as a cause of bio-fertilization (Hassan 2009; Zaki et al. 2012). Moreover, *Azotobacter* may increase the concentration of beneficial soil organisms and the plant nutrients availability in soil (Hamidi et al. 2008).

The growth parameters were recorded at plant harvest stage to investigate the effect of bio-fertilization with microorganisms on the roselle growth. Bio-fertilization significantly \((p < 0.05)\) increased the fresh, dry weights plant high, root length, and number of sepals and branch by 7.53, 8.57, 2.61, 4.13, 27.43, and 11.86%, respectively, compared with the untreated plants. These results were similar to that of Youssef, Mady, and Ali (2014) who reported that the bio-fertilization increased the yield of cruciferous vegetables. Kandeel and Sharaf (2003) recorded that the maximum values of plant height and number of branches of *Majorana hortensis* plants were obtained from the treatment of bi-fertilizers in comparison to the non-treated ones. Bio-fertilizers application may be include some hormone substances, that is, gibberellins, auxins and cytokinins (Umesha, Singh, and Singh 2018).

Effect of bio-fertilizer on some photosynthetic pigments

Data in Figure 2 show the effect of bio-fertilization on the photosynthetic pigments. The bio-fertilization with microorganisms significantly \((p < 0.05)\) increased the chlorophyll a, b, and total
by 5.41, 79.67, and 16.45% compared with un-inoculated plants. In the same trend the carotenoid content in the leaf tissues of roselle plants was increased by and 26.10% above the control. The higher total chlorophyll content as well as the higher accumulation of various metabolites may result from enhancing plant growth and biomass production (Hassan 2009 and Kahil, Hassan, and Ali 2017). The chlorophyll contents of roselle leaves were significantly increased due to the bio-fertilizer treatments relative to the control (Kahil, Hassan, and Ali 2017). Bio-fertilizers may stimulate chlorophyll synthesis through encourages pyridoxal enzymes formation, that play an important role in \( \alpha \)-amino levulinic acid synthetase as a primary compound in chlorophyll synthesis (Ramadan, Hassan, and Abdo 2003). These results are supported by other published researches (Hassan 2009; Kahil, Hassan, and Ali 2017; El-Mahdy et al., 2018).

**Effect of bio-fertilizer on nitrogen (N) phosphorus (P), and potassium (K) uptake by roselle plants**

The effect of bio-fertilization on the uptake of N, P, and K was investigated at the full blooming stage and at the plant harvest and the data are shown in Figure 3. The obtained results demonstrated that in the full blooming stage N, P, and K concentrations increased by 11.45, 9.5, and 3.77%, respectively, while the uptake was increased by 15.14, 46.06, and 36.1%, respectively, as compared with the untreated plants. N, P, and K in roselle leaves were significantly \((p < 0.05)\) affected by the bio-fertilizer application at full blooming stage. The obtained results indicated that the contents of those macro elements were significantly increased in the plants inoculated with *Azotobacter chroococcum* and *Azospirillum brasilense* compared with the un-inoculated plants. Similar results were reported by Kahil, Hassan, and Ali (2017) who found that the N, P, and K concentrations were increased and this increment led to promote the growth and yield of roselle plants. This may be due to that microorganisms also produce growth promoting substances resulting in more efficient absorption of nutrients (Umesha, Singh, and Singh 2018). In addition,
the non-symbiotic N₂-fixing bacteria produced adequate amounts of IAA and cytokinins with increasing the surface area per unit root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil on coriander plant (Cocking 2003; Hassan 2009).

The data in Table 2 showed that the N, P, and K concentrations were increased by 9.24, 10.72 and 4.42% respectively as compared with untreated plants. Also, the uptake of the mentioned nutrients was increased by 18.8, 17.81, and 12.75%, respectively as compared with untreated plants. The obtained results indicated that the bio-fertilizers had significant effects on N, P, and K uptake; on the other hand, the lowest values in this respect were obtained in the absence of bio-fertilizer. These results agreed with those obtained by Kandeel and Sharaf (2003) in Majorana plant. These results may be due to the converting of the unavailable forms of nutrient to available forms by the microorganisms in the biofertilizer (Kandeel and Sharaf 2003; Hassan 2009). Soil microorganisms that colonize the rhizosphere may assist plants in the uptake of several vital nutrients, such as P, K, and N from soil (Cocking 2003).

**Effect of bio-fertilizer on some soil chemical characteristics**

Some soil properties i.e., pH, OM as well N, P, and K availability were studied after the harvesting of roselle plants and the data are presented in Table 3. The results indicated that a slight non-significant decrease in the soil pH values was observed as result of bio-fertilization. This may be due to the organic acids produced by bacteria during the mineralization of organic materials (Banerjee et al. 2011; Eissa 2016b; Youssef and Eissa 2017). A slight non-significant increase in the soil organic matter (SOM) was recorded in the soil treated with the bio-fertilizers in comparison to the other treatment (without bio-fertilizers). This may be due to the increasing in vegetative growth and total plant biomass that might have resulted because of the enhanced photosynthesis and better translocation and accumulation of nutrients (Banerjee et al. 2011; Youssef and Eissa 2017). Nutrients uptake and growth of strawberry increased as a result of N-biofertilizer application (Youssef and Eissa 2017).

Available soil N was significantly increased with the inoculation compared to the non-inoculated treatments, this increase was from 80.39 to 91.91 (14.33%). The increase in N
availability in the current study may be due to the increasing in nitrogen fixation through bacterial and increase the native soil nitrogen by the microorganisms in the bio-fertilizer (Das, Dang, and Shivananda 2008; Umesha, Singh, and Singh 2018; Eissa et al. 2018b). In addition, the non-symbiotic N₂-fixing bacteria (Azospirillum) produced adequate amounts of IAA and cytokinins with increasing the surface area per unit of root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil (Valadabadi and Farahani 2011; Weaam et al. 2014). Azotobacter helps to improve plant growth and to increase soil

<table>
<thead>
<tr>
<th>Table 2. Effect of bio-fertilizer on nitrogen (N) phosphorus (P) and potassium (K) concentrations and uptake by roselle plants at plant harvest.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>N concentration</td>
</tr>
<tr>
<td>P concentration</td>
</tr>
<tr>
<td>K concentration</td>
</tr>
<tr>
<td>N Uptake</td>
</tr>
<tr>
<td>P Uptake</td>
</tr>
<tr>
<td>K Uptake</td>
</tr>
</tbody>
</table>

The data were recorded at plant harvest. Means in the same row denoted by different letters are significantly difference according to Duncan’s test at \( p < 0.05 \).

<table>
<thead>
<tr>
<th>Table 3. Effect of bio-fertilizer on some soil chemical properties.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>pH (1:2.5)</td>
</tr>
<tr>
<td>EC (ds/m)</td>
</tr>
<tr>
<td>OM %</td>
</tr>
<tr>
<td>Available N mg kg⁻¹</td>
</tr>
<tr>
<td>Available K mg kg⁻¹</td>
</tr>
<tr>
<td>Available P mg kg⁻¹</td>
</tr>
</tbody>
</table>

The data were recorded at plant harvest. Means in the same row denoted by different letters are significantly difference according to Duncan’s test at \( p < 0.05 \).

Figure 4. Effect of bio-fertilizer on total on yield and quality of roselle sepals. FW = fresh weight of sepals (g plant⁻¹), DW = dry weight sepals (g plant⁻¹). The data were collected at plant harvest. Means denoted by different letters are significantly difference according to Duncan’s test at \( p < 0.05 \).

Availability in the current study may be due to the increasing in nitrogen fixation through bacterial and increase the native soil nitrogen by the microorganisms in the bio-fertilizer (Das, Dang, and Shivananda 2008; Umesha, Singh, and Singh 2018; Eissa et al. 2018b). In addition, the non-symbiotic N₂-fixing bacteria (Azospirillum) produced adequate amounts of IAA and cytokinins with increasing the surface area per unit of root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil (Valadabadi and Farahani 2011; Weaam et al. 2014). Azotobacter helps to improve plant growth and to increase soil
nitrogen through nitrogen fixation by utilizing carbon for its metabolism (Cocking 2003; Umesha, Singh, and Singh 2018). Phosphorus and potassium are also major nutrients for plants and microorganisms but in the current study the soil was moderately available P (8.5 mg kg$^{-1}$) and highly content of K (400 mg kg$^{-1}$). Available soil P and K were not affected by the inoculation in the current study.

Effect of bio-fertilizer on sepal yield and quality of roselle

Data in Figure 4 show the effect of bio-fertilization on the fresh and dry weight of sepals. The bio-fertilization with *Azotobacter + Azospirillum* significantly ($p < 0.05$) increased the fresh and dry weights by 5.89 and 3.55% above the un-treated plants. Increasing the fruit number of roselle plant due to the bio-fertilizer inoculation may attribute to the increment in branch number as shown in the data of Table 4 and Figure 1. In this study, nitrogen fixing bacteria (*Azotobacter + Azospirillum*) promoted roselle yield through the enhancement of plant growth. These results are in agreement with those of Valadabadi and Farahani (2011). Nitrogen fixing bacteria have the ability not only to fix nitrogen but also to release certain phytohormons of GA3 and IAA nature which could stimulate plant growth, absorption of nutrients, and photosynthesis process (Umesha, Singh, and Singh 2018). These results are in accordance with those obtained by (Weaam et al. 2014). Inoculating roselle plants with *Azorobacter* and *Azospirillum* aims to take

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Bio-fertilizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh weight</td>
<td>290.72 b</td>
<td>312.59 a</td>
</tr>
<tr>
<td>Dry weight</td>
<td>62.85 b</td>
<td>68.23 a</td>
</tr>
<tr>
<td>Plant height</td>
<td>119.05 a</td>
<td>122.15 a</td>
</tr>
<tr>
<td>Root length</td>
<td>25.44 b</td>
<td>26.49 a</td>
</tr>
<tr>
<td>Number of sepals/plant</td>
<td>30.49 b</td>
<td>34.10 a</td>
</tr>
<tr>
<td>Number of branches/plant</td>
<td>27.95 b</td>
<td>35.62 a</td>
</tr>
</tbody>
</table>

The data were recorded at plant harvest. Means in the same row denoted by different letters are significantly different according to Duncan’s test at $p < 0.05$.

**Figure 5.** Effect of bio-fertilizer on total anthocyanin (TAC) and total flavonol (TF) content in the sepals of roselle plants. TAC = total anthocyanin and TF = total flavonol. The data were collected at plant harvest. Means denoted by different letters are significantly difference according to Duncan’s test at $p < 0.05$. 

Effect of bio-fertilizer on sepal yield and quality of roselle

Data in Figure 4 show the effect of bio-fertilization on the fresh and dry weight of sepals. The bio-fertilization with *Azotobacter + Azospirillum* significantly ($p < 0.05$) increased the fresh and dry weights by 5.89 and 3.55% above the un-treated plants. Increasing the fruit number of roselle plant due to the bio-fertilizer inoculation may attribute to the increment in branch number as shown in the data of Table 4 and Figure 1. In this study, nitrogen fixing bacteria (*Azotobacter + Azospirillum*) promoted roselle yield through the enhancement of plant growth. These results are in agreement with those of Valadabadi and Farahani (2011). Nitrogen fixing bacteria have the ability not only to fix nitrogen but also to release certain phytohormons of GA3 and IAA nature which could stimulate plant growth, absorption of nutrients, and photosynthesis process (Umesha, Singh, and Singh 2018). These results are in accordance with those obtained by (Weaam et al. 2014). Inoculating roselle plants with *Azorobacter* and *Azospirillum* aims to take
the advantage of their multiple mechanisms rather than their individual effects in the growth promotion, mainly through N nutrition of plants. The inoculation of plants with a mixture of Azorobacter and Azospirillum is more advantage than their individual effects (Weaam et al. 2014).

Figure 5 shows the effect of bio-fertilization on anthocyanin and total flavonol concentrations in roselle sepal. The bio-fertilization significantly ($p < 0.05$) increased anthocyanin and flavonol by 8.44 and 14.27% above the untreated plants. Kahil, Hassan, and Ali (2017) found that anthocyanin concentrations in roselle plants were increased by 16% above the untreated plants as a result of inoculation with Azotobacter chroococcum and Azospirillum brasilense. The sepals’ quality attributes i.e., anthocyanin content of the inoculated plants significantly enhanced in roselle juice compared with the un-inoculated plants (Kahil, Hassan, and Ali 2017). Increasing the anthocyanin content as well as the total flavonol of roselle has been also reported when the bio-fertilizers were applied to date palm (Phoenix dactylifera L.) (Naser et al. 2016).

Conclusion

Azotobacter and Azospirillum are nitrogen fixing bacteria which can be helpful in the development and production of roselle plants. Roselle seeds were inoculated with a bio-fertilizer consists of Azorobacter and Azospirillum in a pot experiment. The results indicated that roselle plants responded significantly to the inoculation with the bio-fertilizer. The bio-fertilization increased the photosynthetic pigments i.e., chlorophyll, carotenoid, total anthocyanin, and total flavonol and nutrients uptake. The inoculation of roselle seeds with Azorobacter and Azospirillum increased the quality and yield of the plant. More studies about the interaction of Azotobacter and Azospirillum with the different environment soil conditions are needed.

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