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ORIGINAL PAPER

# Combined Effect of Prickly Pear Waste Biochar and Azolla on Soil Fertility, Growth, and Yield of Roselle (*Hibiscus sabdariffa* L.) Plants

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## Abstract

Although the use of biochar to promote plant growth has been reported by many researchers, the combined effect of prickly pear waste biochar (BC) and Azolla (AZ) in a field experiment on the roselle plants did yet receive attention. Therefore, the study aims to evaluate the effect of biochar and Azolla extract on the growth, production, and quality of roselle plants. The experiment treatments were in a completely randomized block design with three replicates. Biochar was added at rates of 0, 10, and 20 ton ha$^{-1}$ and AZ was applied at rates of 0, 3, and 6% in addition to a control treatment. Biochar added at high rate (20 ton ha$^{-1}$) significantly increased the fresh and dry weights of sepals by 27.98 and 35.73%, respectively, compared to the control. The corresponding values were significantly increased by 11.89 and 11.85% over the control when Azolla was added at rate of 6%. The interaction effect of both BC and AZ treatments at high rate significantly increased the fresh and dry weight of sepals by 47.16 and 60.59%, respectively, compared to the control. The interaction effect of BC and AZ realized significant effect on soil properties, growth and yield, as well as pigments of roselle plants. This is a good evident means that BC and AZ applications separately or combined are considered promising materials for sustainable organic agriculture and safety food.

**Keywords** Biochar · Azolla · SPAD · Roselle · Plant growth

## 1 Introduction

Roselle (*Hibiscus sabdariffa* L.) plant is one of the most important medicinal and nutritional plants native to Africa and consumed worldwide due to its high value and access to international markets (Sanders et al. 2020). In addition, roselle plant is rich in anthocyanins, organic acids, pectin, phenolic compounds, and vitamins. It is considered an ideal plant in the developed countries like Egypt, and it is drought resistant, relatively easy to cultivate but it requires a lot of labor to deal with due to the difficulty of using mechanization for harvesting (Al-Sayed et al. 2020; Alam et al. 2016).

Nowadays, a great attention is devoted to biochar which is a carbon-rich material formed by thermo-chemically converting plant biomass in an oxygen-deficient environment (McGlashan et al. 2012). It is an important recycling strategy in sustainable development that allows agricultural wastes to be converted into fertilizers or as soil conditioners that improve its properties and fertility (Rekaby et al. 2021; Tenic et al. 2020). Biochar application increases nutrient content, enhances cation exchange capacity, and improves soil structure, diversity of micro-organisms, and ensuring environmental sustainability (Qayyum et al. 2020; Solaiman et al. 2020). Furthermore, plant photosynthetic rate, chlorophyll content, and stomatal conductance were improved by biochar addition (Akhtar et al. 2014; Batool et al. 2015). Consequently, it enhances growth parameters, seed germination, shoot and root lengths, nutrient contents, and crop yield (Ma et al. 2019; Nobile et al. 2020).

On the other hand, biochar has few negative effects such as those related to its high salt content and high acidity, which sometimes leads to undesirable changes especially in the alkaline soil. Also, it has been reported that biochar produced with relatively high temperatures (600–700 °C)

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leads to high proportions of aromatic C and low proportions of hydrogen (H) and oxygen (O) functional groups and consequently low cation exchange capacity (Lehmann and Joseph 2009; Novak et al. 2009).

Azolla is considered one of the most promising bio fertilizers since it has the ability to fix about 30–60% kg N $ha^{-1}$ from atmospheric nitrogen that could replace 25% of nitrogen mineral fertilization (Maswada et al. 2021; Malyan et al. 2019; Kollah et al. 2016). Azolla decomposes through 8–10 days and it releases its N content into soil solution to be available for plant uptakes (Yadav et al. 2014). It has been widely used as a cheap green amendments or bio-fertilizer to supply plants with their N requirements (Abou Hussien et al. 2020; Al-Sayed et al. 2019). Azolla work for availability of macro nutrient that could be changed over time with an average of 8.3% K and 0.6% Mg as well as vitamins production (El-Serafy et al. 2021; Zhang et al. 2018).

Using Azolla or biochar individually or in conjunction with other organic materials enhances plant growth (Sharifi et al. 2019). Combining biochar and Azolla together increases rice yield and nitrogen use efficiency (NUE), reduces chemical fertilizer applications, avoids agricultural pollution, and provides less production costs (Kimani et al. 2021). So far, few studies were conducted to assess the effect of both Azolla and biochar on crop growth and production parameters. We hypothesized that combined both biochar and Azolla represent an important cultivation management option for sustainable agriculture.

Therefore, accordingly, this study aims to (1) evaluate the effect of using Azolla or biochar individual or in conjunction on calyces yield and growth characteristics of roselle plants and (2) to find out the suitable rate of biochar or Azolla that enhance soil properties and roselle plant production.

## 2 Materials and Methods

### 2.1 Experimental Site and Design

A field study was conducted during two successive summer seasons of 2020 and 2021 at a private farm named Hajer Al-Dabayah village southwest Luxor Governorate, Egypt, which is located at 25° 41′ 28.18″ N latitude and 32° 34′ 09.62″ E longitude. The meteorological data of the experimental site are monitored via the Central Lab of Agricultural Climate, Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Giza, Egypt (Table 1). The physico-chemical properties of the soil used in the experiment are listed in Table 2. Roselle (Sobahia 17 dark variety) seeds were obtained from the ARC, Giza, Egypt, and were sown in the field on 26 April of both growing seasons (2020 and 2021). The experimental unit was 2.8 m in length × 1.2 m in width with an area of 3.36 $m^2$ containing

**Table 2** Some physical and chemical properties of studied soil before two successive growing seasons (2020–2021)

| Studied soil | | |
|---|---|---|
| Properties | Before 1st season | Before 2nd season |
| Sand (g $kg^{-1}$) | 750 | 745 |
| Silt (g $kg^{-1}$) | 170 | 174 |
| Clay (g $kg^{-1}$) | 80 | 81 |
| Texture | Sandy loam | Sandy loam |
| Soil reaction (pH) | 7.98 | 7.99 |
| Electrical conductivity (EC) | 1.1 | 1.12 |
| $CaCO_3$ (g $kg^{-1}$) | 47 | 49 |
| Organic matter (g $kg^{-1}$) | 8.2 | 8.5 |
| Available–N (mg $kg^{-1}$) | 21 | 24 |
| Available–P (mg $kg^{-1}$) | 6.2 | 6.6 |
| Available–K (mg $kg^{-1}$) | 221 | 226 |

Each value represents a mean of three replicates

**Table 1** Basic climatic data of the experimental site during the period of the study (April–October 2020–2021)

| | $T_{max}$ (C°) | | $T_{min}$ (C°) | | Relative humidity (%) | | Solar radiation (MJ/$m^2$/day) | | Wind speed $MS^{-1}$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| April | 32.30 | 34.77 | 15.07 | 15.69 | 25.34 | 19.02 | 22.57 | 27.62 | 3.00 | 2.99 |
| May | 38.08 | 40.12 | 20.92 | 21.24 | 19.91 | 14.80 | 27.41 | 28.71 | 3.35 | 3.25 |
| June | 40.80 | 40.62 | 23.22 | 23.10 | 18.05 | 18.86 | 27.89 | 29.12 | 3.22 | 4.05 |
| July | 40.89 | 41.09 | 24.78 | 25.11 | 20.41 | 20.95 | 29.38 | 27.98 | 3.59 | 3.69 |
| August | 41.00 | 41.58 | 24.50 | 24.39 | 21.48 | 20.63 | 28.13 | 26.62 | 3.84 | 3.43 |
| September | 41.85 | 38.69 | 24.27 | 22.39 | 23.02 | 28.02 | 27.01 | 23.94 | 3.19 | 3.84 |
| October | 37.78 | 35.35 | 20.89 | 18.95 | 27.19 | 30.87 | 24.27 | 20.25 | 3.14 | 3.03 |

$T_{max}$, maximum temperature; $T_{min}$, minimum temperature; *MJ/m²*, mega joules per square meter; *MS.⁻¹*, meter/second two rows 60 cm apart with three hills (40 cm apart), with a total density of 41,667 plants ha$^{-1}$. Fifteen days after sowing (DAS), the seedlings were thinned to one plant per hill. The experiment was laid in a randomized complete block with three replicates. The main plot was assigned to biochar (BC) application at rate of 0, 10, and 20 ton ha$^{-1}$ while azolla (AZ) solution was sprayed on plants at rate of 0, 3, and 6% as subplot.

In the early morning, the tested rates of Azolla solutions were sprayed at 50, 80, and 110 DAS for related plot. Distilled water was sprayed on plants in the same times in the control plot. All agriculture practices were performed according to the Egyptian Ministry of Agriculture.

## 2.2 Azolla Extract Preparation

Azolla (*Azolla pinnata* L.) plants were obtained from Soil, Water and Environment Research Institute, ARC, Giza. One kilogram of Azolla plants was soaked in 1 l of ethanol (90% conc.) for 24 h then mixed well with blender. The mixture was filtered twice through two layers of gauze cloth. The obtained solution was considered as 100% concentrate of Azolla plants extract. Three and 6 ml of this concentration were taken and diluted with 97 and 94 ml distilled water to obtain 3 and 6% concentrations, respectively; then, they were kept in the refrigerator at 4 °C until use. Tween 20 at 0.1% (v/v) was used as a surfactant according to Yasmeen et al. (2013). The characteristics of the Azolla solution are shown in Table 3.

## 2.3 The Preparation Procedure of Biochar

The prickly pear fruit wastes (its peels) were collected, air-dried after being cut into small pieces (less than 5 cm), and then oven-dried at 70 °C for 24 h. The raw material was pyrolyized in a muffle furnace at 350 °C for 3 h in limited oxygen conditions. After that, it was passed through a 2-mm diameter stainless steel sieve before mixing it with the soil. The properties of biochar are shown in Table 3.

**Table 3** Chemical composition of the used Azolla and biochar

| Property | Azolla | Biochar |
|---|---|---|
| Soil reaction (pH) | 7.5 | 8.12 |
| Electrical conductivity (EC) | 1.98 | 5.14 |
| Organic matter (g kg$^{-1}$) | 620 | 630 |
| Total nitrogen (g kg$^{-1}$) | 34.00 | 23 |
| Total phosphorous (g kg$^{-1}$) | 17.30 | 5.1 |
| Total potassium(g kg$^{-1}$) | 22.00 | 11 |
| Total Mg (g kg$^{-1}$) | 15.00 | - |
| Total Mn (g kg$^{-1}$) | 1.40 | - |
| Total Fe( g kg$^{-1}$) | 1.80 | - |

Each value represents a mean of three replicates

## 2.4 Soil, Plant, Biochar, and Azolla Analysis

Some physical and chemical properties of the tested soils were determined according to Burt (2004). Soil texture was determined by the pipette as described by Page et al. (1982). The soil reaction was measured potentiometrically in soil (Page et al. 1982) using a digital pH meter (Hanna Instruments pH 211, Romania). The electrical conductivity (EC) was determined using the salt bridge by an EC meter (Jenway 4510 England) (Burt 2004). Calcium carbonate was determined according to Burt (2004). Available phosphorus (P) was measured according to the method describe by Olsen and Sommers (1982). Available potassium (K) was extracted by 1 N ammonium acetate solution measured by the flame photometer according to (Jackson 1973). Available nitrogen was extracted with 1% $K_2SO_4$ using a micro Kjeldahl's method (Jackson 1973). Soil organic matter (SOM) concentration was determined by oxidization with $K_2Cr_2O_7$ and $H_2SO_4$ (Jackson 1973).

Biochar and Azolla samples (2.0 g) were digested with $H_2O_2$ and $H_2SO_4$. The total N, P, and K concentrations were measured in the digest extract. To measure nutrient concentrations in roselle shoots, a mixture of 7:3 ratio of sulfuric to perchloric acids was used to digest the dried ground plant material. The total N, P, and K determined were described by Burt (2004). The nutrient uptake of N, P, and K was calculated by the following formula: (Total N, P, and K content × dry matter)/100. Chlorophyll contents from fully developed leaves were determined using a portable chlorophyll meter (SPAD-502-m Konica Minolta, Inc., Tokyo, Japan). Total anthocyanins (TAC) and total flavonoids (TF) were extracted by adding 10 ml (8.5 ml ethanol 96% + 1.5 ml HCl 1.5 M) to 1 g of dried sepals according to Lees and Francis (1971).

### 2.4.1 Relative Water Content (RWC)

To determine the relative water content (RWC) of ripe leaves at harvest, random leave samples from each treatment were weighed directly to calculate the fresh weight (FW) then soaked in water in test tube in the dark for 24 h. They were blotted dry with filter paper and weighed to calculate their turgid weight (TW). The leaves oven dried at 70 °C for 48 h to measure their dry weight (DW). The leaf RWC was estimated according to Smart and Bingham (1974) using the following equation:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

### 2.5 Data Analysis

The analysis of variance (two-way ANOVA) and Duncan's multiple range tests at 5% level of probability were performed to distinct the significant differences among the treatments. The statistical analyses were performed using Costat software (Steel and Torrie 1996).

## 3 Results

### 3.1 Soil Properties

The effect of biochar (BC) and Azolla (AZ) application on soil reaction (pH), soil salinity (EC), and soil organic matter (SOM) are shown in Table 4. Generally, all treatments realized significant improvement of soil properties compared to the control. On average basis of both seasons, a slight change in pH values as a result of adding the high biochar rate BC2 while it significantly increased the EC and SOM by about 21 and 78%, respectively, compared to the control treatment. The addition of AZ showed insignificant effect on soil properties. The effect of biochar alone and the combined biochar and Azolla increased EC values and SOM content more than 24 and 77%, respectively, compared to the control.

### 3.2 Nutrient Availability and Their Uptake

Nitrogen (N), phosphorous (P), and potassium (K) availability and their uptake were significantly ($P < 0.05$) increased due to adding BC and/or AZ in both seasons (Tables 5 and 6). On average basis of both seasons, the available N, P, and K increased about 41, 46, and 35%, respectively, as a result of adding the high rate of BC. Also, the uptake of N, P, and K were significantly ($P < 0.05$) increased by 100, 64, and 70%, respectively, due to applying the high rate of BC compared to the control. Regarding AZ addition, the N, P, and K availabilities were significantly ($P < 0.05$) increased almost 57, 8, and 0.52%, respectively, compared to the control. Also, their uptake were significantly ($P < 0.05$) increased 25, 21, and 24%, respectively, compared to the control. When addition BC with AZ sprayed on plants, the N, P, and K availability and their uptake were positively affected. N, P, and K availability increased about 52, 51, and 37%, respectively, while their uptake increased nearly 144, 102 and 109%, respectively, compared to the control.

### 3.3 Some Growth Parameters

The results showed that all treatments were significantly ($P < 0.05$) increased the growth parameters as biochar and Azolla rates increased and the increases were more

**Table 4** Impact of different rates of biochar and or/Azolla on the physicochemical properties of soil analyzed after harvesting of the roselle plant

| Azolla% | First season | | | | Second season | | | |
|---|---|---|---|---|---|---|---|---|
| | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B |
| pH | | | | | | | | |
| 0 | 8.13 ± 0.04d | 8.18 ± 0.01bcd | 8.2 ± 0.01ab | 8.18A | 8.10 ± 0.03c | 8.25 ± 0.01b | 8.30 ± 0.01ab | 8.21A |
| 3 | 8.13 ± 0.01 cd | 8.17 ± 0.03bcd | 8.24 ± 0.01ab | 8.18A | 8.08 ± 0.02c | 8.25 ± 0.03b | 8.31 ± 0.01a | 8.21A |
| 6 | 8.12 ± 0.01 cd | 8.19 ± 0.01abc | 8.25 ± 0.01a | 8.19A | 8.05 ± 0.02c | 8.27 ± 0.01ab | 8.33 ± 0.01a | 8.22A |
| Biochar mean A | 8.13C | 8.18B | 8.24A | | 8.08C | 8.25B | 8.31A | |
| EC (dS $m^{-1}$) | | | | | | | | |
| 0 | 0.66 ± 0.01d | 0.70 ± 0.03 cd | 0.77 ± 0.03ab | 0.71A | 0.65 ± 0.02d | 0.75 ± 0.02bc | 0.82 ± 0.07ab | 0.74A |
| 3 | 0.68 ± 0.03d | 0.71 ± 0.02bcd | 0.75 ± 0.03abc | 0.71A | 0.64 ± 0.03d | 0.77 ± 0.03abc | 0.83 ± 0.03ab | 0.75A |
| 6 | 0.68 ± 0.04d | 0.73 ± 0.06abcd | 0.78 ± 0.02a | 0.73A | 0.67 ± 0.02 cd | 0.76 ± 0.01abc | 0.85 ± 0.01a | 0.76A |
| Biochar mean A | 0.67C | 0.71B | 0.77A | | 0.66C | 0.76B | 0.84A | |
| OM (g $kg^{-1}$) | | | | | | | | |
| 0 | 6.42 ± 0.22c | 9.56 ± 0.02b | 10.95 ± 0.04a | 8.97A | 6.36 ± 0.21c | 10.34 ± 0.03b | 11.71 ± 0.06a | 9.47A |
| 3 | 6.38 ± 0.16c | 9.57 ± 0.39b | 10.98 ± 0.41a | 8.98A | 6.35 ± 0.09c | 10.34 ± 0.31b | 11.72 ± 0.46a | 9.47A |
| 6 | 6.37 ± 0.15c | 9.57 ± 0.12b | 10.99 ± 0.09a | 8.97A | 6.34 ± 0.23c | 10.35 ± 0.07b | 11.73 ± 0.04a | 9.47A |
| Biochar mean A | 6.39C | 9.56B | 10.97A | | 6.35C | 10.34B | 11.72A | |

*BC0, BC1, and BC2*, biochar at rates of (0, 10 and 20 t $ha.^{-1}$); *pH*, soil reaction; *EC*, electrical conductivity; *OM*, organic matter. All values are the mean of three replicate analysis ± standard error. Means in each column followed by the same letters are not significantly different ($P < 0.05$) by Duncan's multiple range tests

**Table 5** Impact of biochar and or/Azolla on the different nutrient availability in roselle plants

| Azolla % | First season | | | | Second season | | | |
|---|---|---|---|---|---|---|---|---|
| | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B |
| N (mg kg$^{-1}$) | | | | | | | | |
| 0 | 28.50 ± 1.73d | 33.70 ± 0.61 cd | 39.60 ± 1.01 ab | 33.93A | 28.23 ± 1.55e | 34.50 ± 0.55 cd | 40.23 ± 1.00ab | 34.32A |
| 3 | 31.20 ± 0.70 cd | 34.67 ± 1.88bc | 41.33 ± 2.77a | 35.73A | 29.67 ± 0.81e | 35.37 ± 1.88bc | 41.98 ± 2.75a | 35.67A |
| 6 | 29.90 ± 1.97 cd | 35.55 ± 1.90bc | 42.87 ± 0.92a | 36.11A | 28.60 ± 2.06de | 36.21 ± 1.92bc | 43.48 ± 0.87a | 36.09A |
| Biochar mean A | 29.87C | 34.64B | 41.27A | | 28.83C | 35.36B | 41.89A | |
| P (mg kg$^{-1}$) | | | | | | | | |
| 0 | 6.61 ± 0.02c | 7.26 ± 0.14c | 9.010 ± 0.04ab | 7.63B | 6.27 ± 0.35d | 7.88 ± 0.14c | 9.66 ± 0.07ab | 7.93B |
| 3 | 6.66 ± 0.11c | 8.46 ± 0.03b | 9.47 ± 0.19a | 8.20A | 6.63 ± 0.08d | 9.07 ± 0.03b | 10.11 ± 0.16a | 8.60A |
| 6 | 6.77 ± 0.12c | 8.47 ± 0.74b | 9.46 ± 0.28a | 8.23A | 6.73 ± 0.15d | 9.09 ± 0.71b | 10.09 ± 0.24a | 8.63A |
| Biochar mean A | 6.68C | 8.06B | 9.32A | | 6.54C | 8.68B | 9.95A | |
| K (mg kg$^{-1}$) | | | | | | | | |
| 0 | 279.51 ± 3.36c | 290.79 ± 1.42b | 378.33 ± 4.06a | 316.21A | 279.16 ± 3.04c | 292.01 ± 1.42b | 379.54 ± 4.05a | 316.90A |
| 3 | 280.33 ± 2.03c | 290.82 ± 4.54b | 378.56 ± 10.78a | 316.57A | 280.31 ± 2.03c | 292.15 ± 4.47b | 379.81 ± 10.79a | 317.42A |
| 6 | 281.31 ± 1.22c | 293.05 ± 2.08b | 378.99 ± 8.10a | 317.79A | 281.25 ± 1.22c | 294.32 ± 2.08b | 380.24 ± 8.16a | 318.60A |
| Biochar mean A | 280.39C | 291.56B | 378.63A | | 280.24C | 292.83B | 379.86A | |

*BC0, BC1, and BC2*, biochar at rates of (0, 10, and 20 t ha.$^{-1}$). All values are the mean of three replicate analysis ± standard error. Means in each column followed by the same letters are not significantly different ($P < 0.05$) by Duncan's multiple range tests

**Table 6** Impact of biochar and/or Azolla on the different nutrient uptake in roselle plants

| Azolla % | First season | | | | Second season | | | |
|---|---|---|---|---|---|---|---|---|
| | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B |
| N uptake (g kg$^{-1}$) | | | | | | | | |
| 0 | 7.52 ± 0.30e | 10.97 ± 0.43d | 14.11 ± 0.10bc | 10.87C | 7.16 ± 0.32f | 11.94 ± 0.58d | 16.13 ± 0.26b | 11.75C |
| 3 | 8.26 ± 0.47e | 12.95 ± 0.41c | 15.36 ± 0.12b | 12.19B | 8.20 ± 0.55ef | 14.11 ± 1.17c | 18.02 ± 0.63a | 13.44B |
| 6 | 8.79 ± 0.61e | 14.55 ± 0.18b | 17.00 ± 0.19a | 13.44A | 9.43 ± 0.86e | 15.94 ± 0.43b | 19.47 ± 1.16a | 14.95A |
| Biochar mean A | 8.19C | 12.82B | 15.49A | | 8.27C | 14.00B | 17.87A | |
| P uptake (g kg$^{-1}$) | | | | | | | | |
| 0 | 0.75 ± 0.02f | 1.01 ± 0.04d | 1.26 ± 0.04c | 1.01C | 0.69 ± 0.02f | 1.02 ± 0.05d | 1.23 ± 0.04bc | 0.98C |
| 3 | 0.83 ± 0.02ef | 1.19 ± 0.04c | 1.37 ± 0.02b | 1.13B | 0.86 ± 0.02e | 1.18 ± 0.02c | 1.32 ± 0.03b | 1.12B |
| 6 | 0.86 ± 0.04e | 1.25 ± 0.04c | 1.49 ± 0.02a | 1.20A | 0.89 ± 0.05e | 1.28 ± 0.04bc | 1.47 ± 0.03a | 1.21A |
| Biochar mean A | 0.81C | 1.15B | 1.38A | | 0.81C | 1.16B | 1.34A | |
| K uptake (g kg$^{-1}$) | | | | | | | | |
| 0 | 8.08 ± 0.04i | 11.35 ± 0.32f | 13.72 ± 0.96d | 11.05C | 8.68 ± 0.43 g | 12.15 ± 0.21de | 14.50 ± 0.97bc | 11.78C |
| 3 | 8.91 ± 0.53 h | 12.75 ± 0.50e | 15.71 ± 0.057b | 12.46B | 9.77 ± 0.54 fg | 13.52 ± 0.45 cd | 16.45 ± 0.56ab | 13.25B |
| 6 | 9.82 ± 0.32 g | 14.20 ± 0.50c | 17.25 ± 1.00a | 13.76A | 10.78 ± 0.34ef | 14.99 ± 0.49bc | 17.85 ± 1.01a | 14.54A |
| Biochar mean A | 8.94C | 12.77B | 15.56A | | 9.74C | 13.56B | 16.27A | |

*BC0, BC1, and BC2*, biochar at rates of (0, 10, and 20 t ha.$^{-1}$). All values are the mean of three replicate analysis ± standard error. Means in each column followed by the same letters are not significantly different ($P < 0.05$) by Duncan's multiple range tests

**Table 7** Impact of different rates of biochar and/or Azolla on the shoot parameters of roselle plants

| Azolla % | First season | | | | Second season | | | |
|---|---|---|---|---|---|---|---|---|
| | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B |
| Fresh weight plant$^{-1}$ (g) | | | | | | | | |
| 0 | 202.40 ± 2.00 g | 224.20 ± 2.61e | 240.77 ± 1.56c | 222.46C | 199.20 ± 2.00 h | 226.57 ± 2.36f | 245.00 ± 1.46c | 223.59C |
| 3 | 207.30 ± 0.78f | 228.60 ± 2.21e | 248.33 ± 1.59b | 228.08B | 208.73 ± 0.74 g | 231.43 ± 2.19e | 253.23 ± 1.53b | 231.13B |
| 6 | 210.73 ± 2.08f | 235.40 ± 2.01d | 255.60 ± 0.69a | 233.91A | 212.60 ± 1.99 g | 239.20 ± 2.08d | 261.27 ± 0.58a | 237.69A |
| Biochar mean A | 206.81C | 229.40B | 248.23A | 206.81C | 206.84C | 232.40B | 253.17A | 206.84C |
| Dry weight plant$^{-1}$ (g) | | | | | | | | |
| 0 | 83.13 ± 2.74c | 89.77 ± 1.47b | 89.77 ± 1.47b | 79.32B | 70.40 ± 1.32e | 88.30 ± 2.91c | 93.87 ± 1.40bc | 84.19B |
| 3 | 89.97 ± 2.82b | 93.00 ± 1.59ab | 93.00 ± 1.59ab | 84.52A | 76.90 ± 1.99d | 94.73 ± 2.58ab | 96.50 ± a1.51b | 89.38A |
| 6 | 92.77 ± 1.13ab | 97.97 ± 1.38a | 97.97 ± 1.38a | 87.76A | 79.20 ± 1.99d | 96.97 ± 1.47ab | 100.63 ± 1.13a | 92.26A |
| Biochar mean A (BC) | 88.62B | 93.58A | 93.58A | | 75.50C | 93.33B | 97.00A | |
| Plant height plant$^{-1}$ (cm) | | | | | | | | |
| 0 | 87.83 ± 1.59e | 94.87 ± 2.05 cd | 112.07 ± 0.96b | 98.26C | 86.10 ± 1.57e | 100.07 ± 1.74 cd | 116.33 ± 1.60b | 100.83C |
| 3 | 91.331.68 ± de | 98.83 ± 2.45c | 118.90 ± 1.63a | 103.02B | 95.43 ± 1.96d | 104.57 ± 2.73c | 124.90 ± 0.71a | 108.30B |
| 6 | 93.97 ± 2.12 cd | 110.73 ± 0.71b | 122.33 ± 2.33a | 109.01A | 98.43 ± 2.07 cd | 113.13 ± 2.95b | 126.07 ± 3.58a | 112.54A |
| Biochar mean A | 91.04C | 101.48B | 117.77A | | 93.32C | 105.92B | 122.43A | |

*BC0, BC1, and BC2*, biochar at rates of (0, 10, and 20 t ha.$^{-1}$). All values are the mean of three replicate analysis ± standard error. Means in each column followed by the same letters are not significantly different ($P < 0.05$) by Duncan's multiple range tests

**Table 8** Impact of different rates of biochar and/or Azolla on the root parameters of roselle plants

| Azolla % | First season | | | | Second season | | | |
|---|---|---|---|---|---|---|---|---|
| | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B |
| Root length plant$^{-1}$ (cm) | | | | | | | | |
| 0 | 17.00 ± 1.15c | 24.33 ± 0.88a | 24.67 ± 1.45a | 22.00B | 15.13 ± 0.90c | 25.83 ± 0.84a | 26.07 ± 1.28a | 22.34A |
| 3 | 18.33 ± 1.45bc | 24.67 ± 0.88a | 25.67 ± 0.67a | 22.89AB | 16.67 ± 1.30bc | 25.90 ± 0.96a | 27.07 ± 0.87a | 23.21A |
| 6 | 20.67 ± 0.88b | 25.00 ± 0.00a | 26.00 ± 0.58a | 23.89A | 19.93 ± 1.64b | 26.23 ± 0.09a | 26.80 ± 1.50a | 24.32A |
| Biochar mean A | 18.67B | 24.67A | 25.44A | | 17.24B | 25.99A | 26.64A | |
| Root fresh weight plant$^{-1}$ (g) | | | | | | | | |
| 0 | 22.40 ± 2.08d | 27.00 ± 0.44d | 39.90 ± a1.22b | 29.77B | 23.01 ± 0.56f | 29.37 ± 0.52d | 41.03 ± 0.27ab | 31.14C |
| 3 | 23.93 ± 1.52d | 33.43 ± 2.22c | 41.47 ± 1.71a | 32.94A | 25.23 ± 1.44ef | 33.65 ± 1.40c | 42.77 ± 0.26a | 33.88B |
| 6 | 26.40 ± 0.46d | 35.40 ± 2.40bc | 41.50 ± 2.21a | 34.43A | 28.33 ± 0.55de | 37.90 ± 2.72b | 42.97 ± 0.84a | 36.40A |
| Biochar mean A | 24.24C | 31.94B | 40.96A | | 25.52C | 33.64B | 42.26A | |
| Root dry weight plant$^{-1}$ (g) | | | | | | | | |
| 0 | 7.97 ± 1.84e | 12.47 ± 0.49 cd | 14.13 ± 0.87abc | 11.52B | 7.67 ± 1.22d | 14.23 ± 0.49b | 14.77 ± 1.48ab | 12.22B |
| 3 | 8.60 ± 0.57e | 12.72 ± 1.76bcd | 15.15 ± 0.60ab | 12.16B | 8.30 ± 0.21 cd | 13.76 ± 0.99b | 16.52 ± 1.46ab | 12.86B |
| 6 | 11.13 ± 0.15d | 13.80 ± 1.01abc | 15.86 ± 1.02a | 13.60A | 10.63 ± 0.81c | 15.70 ± 0.96b | 18.09 ± 1.34a | 14.81A |
| Biochar mean A | | 9.23C | 13.00B | 15.05A | 8.87C | 14.56B | 16.46A | |

*BC0, BC1, and BC2*, biochar at rates of (0, 10, and 20 t ha.$^{-1}$). All values are the mean of three replicate analysis ± standard error. Means in each column followed by the same letters are not significantly different ($P < 0.05$) by Duncan's multiple range tests

**Table 9** Impact of different rates of biochar and/or Azolla on some morphological traits of roselle plants

| Azolla % | First season | | | | Second season | | | |
|---|---|---|---|---|---|---|---|---|
| | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B |
| Stem diameter (cm) | | | | | | | | |
| 0 | 0.83±0.15b | 1.13±0.12ab | 1.23±0.09ab | 1.07A | 0.73±0.15d | 1.34±0.13bc | 1.60±0.06ab | 1.23A |
| 3 | 0.93±0.23ab | 1.17±0.03ab | 1.27±0.09a | 1.12A | 1.03±0.23 cd | 1.54±0.12ab | 1.77±0.09a | 1.44A |
| 6 | 1.03±0.07ab | 1.17±0.12ab | 1.33±0.09a | 1.18A | 1.24±0.12bc | 1.40±0.06abc | 1.65±0.08ab | 1.43A |
| Biochar mean A | 0.93B | 1.16A | 1.28A | | 1.00C | 1.43B | 1.67A | |
| Leaf area ($mm^2$) | | | | | | | | |
| 0 | 3547.86±117.10c | 3845.98±247.98bc | 4359.22±298.23ab | 3917.68A | 3545.44±117.27c | 3850.31±247.69bc | 4369.09±298.18ab | 3921.62A |
| 3 | 3650.66±143.63c | 4082.56±139.56abc | 4463.76±217.68a | 4065.67A | 3653.88±143.48c | 4088.21±139.68abc | 4479.09±217.39a | 4073.73A |
| 6 | 3756.93±128.97c | 4130.59±67.44abc | 4471.22±94.12a | 4119.57A | 3760.34±128.64c | 4139.74±68.21abc | 4486.96±93.49a | 4129.01A |
| Biochar mean A | 3651.82C | 4019.7B | 3651.82A | | 3653.22C | 4026.09B | 4445.05A | |

*BC0, BC1, and BC2*, biochar at rates of (0, 10, and 20 t $ha.^{-1}$). All values are the mean of three replicate analysis ± standard error. Means in each column followed by the same letters are not significantly different ($P<0.05$) by Duncan's multiple range tests

pronounced with combined application of BC and AZ (Tables 7, 8, 9, and 10). On the average basis of both seasons, the high rate of biochar application increased the fresh weight, dry weight, and plant height by 21, 31, and 30%, respectively, over the control. Also, the stem diameter, leaf area, and RWC content increased almost by 52, 21, and 32%, respectively, above the control treatment. Azolla foliar application enhanced the growth parameters since the fresh weight, dry weight, and plant height increased around 5, 10, and 11%, respectively, at the high rate (6%) of AZ foliar application. The combined application of BC and AZ augmented the growth parameters of roselle plants. Adding BC and AZ at high rate increased fresh weight, dry weight, and plant height roughly by 28, 46, and 43%, respectively, compared to the control. The same trend was observed with the root parameters sic they were significantly increased as a result of adding biochar and/or Azolla.

### 3.4 Physiological Parameters of Roselle Plants

The high rate of biochar application significantly increased chlorophyll, total anthocyanins (TAC), and total flavonoids (TF) nearby 16, 40, and 62%, respectively, over the control (Fig. 1a–c).

Also, AZ sprayed at high rate significant increased chlorophyll, TAC, and TF almost by 4, 6, and 12%, respectively,

**Table 10** Impact of different rates of biochar and or/Azolla on relative water content (RWC) and sepals number as morphological traits of roselle plants

| Azolla % | First season | | | | Second season | | | |
|---|---|---|---|---|---|---|---|---|
| | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B | $BC_O$ | $BC_1$ | $BC_2$ | Azolla mean B |
| RWC% | | | | | | | | |
| 0 | 62.27±1.13d | 73.34±2.12bc | 83.16±0.81a | 72.92B | 14.00±1.15d | 16.33±0.88 cd | 23.00±1.15b | 17.78B |
| 3 | 64.43±1.47d | 73.63±0.54bc | 83.63±1.81a | 73.90B | 14.33±1.20 cd | 17.67±0.88c | 26.33±1.20a | 19.44B |
| 6 | 71.00±1.12c | 75.52±1.02b | 83.73±2.15a | 76.75A | 15.00±1.15 cd | 22.67±0.88b | 27.33±0.88a | 21.67A |
| Biochar mean A | 65.90C | 74.17B | 83.51A | | 14.44C | 18.88B | 25.56A | |
| Number of sepals $plant^{-1}$ | | | | | | | | |
| 0 | 60.90±1.31d | 76.07±1.91bc | 89.06±0.98a | 75.35B | 12.67±0.88d | 24.00±1.73c | 30.00±1.00ab | 22.22B |
| 3 | 64.47±1.27d | 77.03±0.17bc | 90.53±1.55a | 77.34B | 17.33±1.45d | 28.00±1.73bc | 35.33±2.33a | 26.89A |
| 6 | 72.77±1.10c | 80.29±1.13b | 91.40±2.39a | 81.49A | 18.33±1.20d | 30.67±1.86ab | 34.67±2.60a | 27.89A |
| Biochar mean A | 66.04C | 77.80B | 90.33A | | 16.11C | 27.56B | 33.33A | |

*BC0, BC1, and BC2*, biochar at rates of (0, 10, and 20 t $ha.^{-1}$); *RWC*, relative water content. All values are the mean of three replicate analysis ± standard error. Means in each column followed by the same letters are not significantly different ($P<0.05$) by Duncan's multiple range tests

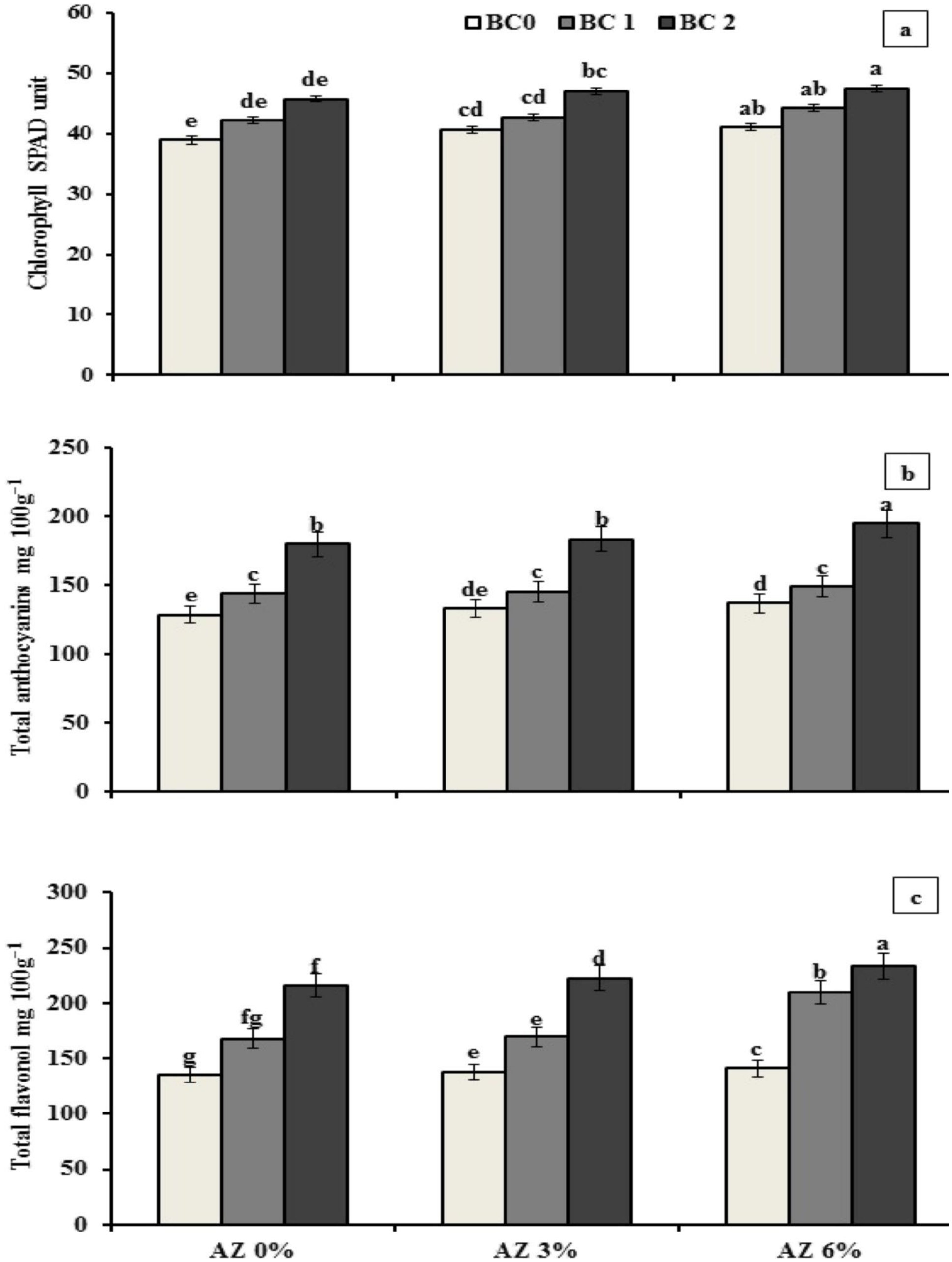


**Fig. 1** Effect of biochar and/or Azolla on chlorophyll SPAD unit (**a**), total anthocyanins (**b**), and total flavonol (**c**) of roselle plants on the average basis of the two seasons. BC0, BC1, and BC2, biochar at rates of (0, 10, and 20 t ha.$^{-1}$); AZ 0%, AZ 3%, and AZ 6%, Azolla at rates of (0, 3, and 6%). Means in each column followed by the same letters are not significantly different ($P < 0.05$) by Duncan's multiple range test

compared to the control treatment. The combined application of BC and sprayed AZ on roselle plants increased chlorophyll, TAC, and TF about by 22, 51, and 72%, respectively, compared to the control treatment.

### 3.5 Yield Components of Roselle Plants

On the average basis of both seasons, the plant yield parameters were improved via high rate of biochar application since sepals no./plant, sepals fresh and dry weight increased nearly by 92, 28, and 35%, respectively, over the control (Fig. 2a and b and Table 10). Also, the fruit number of sepals/plant, sepals fresh, and dry weight increased almost by 23, 11, and 11%, respectively, as a result of sprayed AZ at high rate compared to the control. The application of BC and sprayed AZ on roselle plants increased fruit number, sepals fresh, and dry weight about by 132, 47, and 60%, respectively, compared to the control treatment.

## 4 Discussion

Biochar application changed soil properties such as pH, EC, organic matter, and available N, P, and K as well as their uptake. This might be due to biochar is alkaline material in biochar may explain the higher pH in the biochar treatments

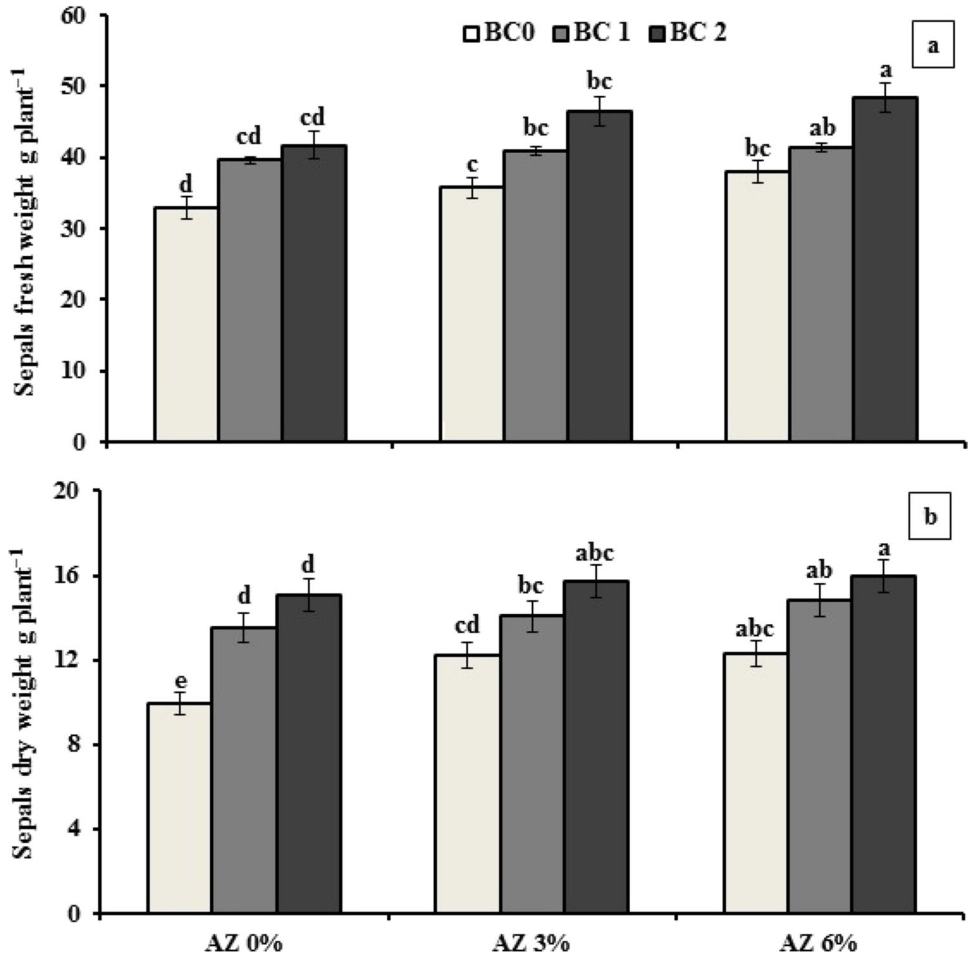


**Fig. 2** Effect of biochar and/or Azolla on the fresh (**a**) and dry (**b**) weight of sepal's yield on the average basis of the two seasons. BC0, BC1, and BC2, biochar at rates of (0, 10, and 20 t ha.$^{-1}$); AZ 0%, AZ 3%, and AZ 6%, Azolla at rates of (0, 3, and 6%). Means denoted by the same letter indicate no significant difference according to Duncan's tests at $p < 0.05$

(Kookana et al. 2011). Also, biochar to the soil is associated with active pH-dependent functional groups, such as OH and COOH that can be raising its pH (Alkharabsheh et al. 2021; Cheng and Lehmann 2009; Weber and Quicker 2018).

The increases of EC value might be due to the high salt content of biochar material. For biochar-treated soils, more mineral ions, such as $Ca^{2+}$, $K^{+}$, and $Mg^{2+}$, were dissolved in soil solution, which was supported by the higher soil salinity (Xu et al. 2016).

Also, biochar application increased soil organic matter (SOM) content that could increase soil water holding capacity (WHC) and enhance nutrient availability such as N, P, and K (Yuan et al. 2016). Agegnehu et al. (2015) indicated that biochar application increased soil organic matter (SOM) by 23.5% over the control. In addition, Murad et al. (2021) noticed that adding biochar at a rate of 4% increased organic matter by 1.67% over the control treatment.

The availability of N, P, and K nutrient as a result of biochar addition can be attributed to its high nutritional supply as direct effect and also as indirect slow release effect of these nutrients (Lei and Zhang 2013; Zheng et al. 2017). Liu et al. (2021) mentioned that biochar application at rate of 2% increased available N, P, and K by 24, 37, and 19%, respectively, to be uptake by roselle plants. Similar results were obtained by Guo et al. (2020), Villagra-Mendoza et al. (2021), Zheng et al. (2021).

The usefulness of Azolla as a good organic fertilizer to improve agricultural production and the environmental sustainability through its ability to reform biological nitrogen reduce fertilizer leaching in addition to its high content of nutrients and vitamins, which leads to enhanced plant growth (Maham et al. 2020).

Improvement nutrients uptake especially N due to the Azolla sprayed on roselle plants might be due to increasing available nitrogen via atmospheric N fixation as well as its wealthy nutrients in available form that easy to be absorbed through leave stomata. The increased nutritional content in roselle plant and pigment content in plant leaves as a result of spraying Azolla could be due to its mineral richness of N, P, K, Mg, Fe, and Mn (Abou-Sreea et al. 2021). Azolla is a plentiful source of macro-and micronutrients crude protein, growth-promoting cytokinins, jasmonic acid, salicylic acid, and vitamins (de Vries et al. 2018; Shaltout et al. 2012; Stirk and Van Staden 2003).

However, the conjunction use of BC and AZ resulted in a significant increase in yield and quality via improvement soil properties, increasing soil organic matter which leads to soil carbon-rich that enhances soil microorganisms and nutrient availability resulting higher plant yield and its quality (Eissa 2019; Ghadimi et al. 2021; Kabiri et al. 2021). Also, combined application of biochar and Azolla improved the growth features of rosemary plants in calcareous soils of arid and semiarid regions (Sadegh Kasmaei et al. 2019). The use of Azolla improved the amount of chlorophyll as well as the photosynthesis process in Beta vulgaris plant (de Bever et al. 2013). This improvement may be due to Azolla sprayed levels on plant leaves increases the metabolites and chlorophyll synthesis that enhance photosynthesis process, which improves yield components and quality by increasing anthocyanins and flavonoids synthesis (Maswada et al. 2021). The chlorophyll content of safflower plants growing in saline-sodic soils was enhanced by 3% using Azolla as compost (Sharifi et al. 2019). The application of Azolla increased significant values of the dry biomass, growth chlorophyll, fruit number, and weight (Youssef et al. 2021). Azolla is high nutritional and organic matter content boosted its potential to improve soil quality and nutrient availability, resulting in considerable squash fruit growth and quality (Abou Hussien et al. 2020).

## 5 Conclusion

Both biochar application and Azolla sprayed on roselle plants improved soil properties, increased nutrients availability and their uptake, and significantly increased roselle growth, yield, and quality. These increases varied according to biochar and/or Azolla application rates. The addition of biochar and Azolla realized an important role in nutrient availability, metabolites, and chlorophyll synthesis and improves photosynthesis process, which is reflected on the yield, yield components, and its quality. Therefore, applying biochar as soil organic amendments at rate of 20 ton ha$^{-1}$ combined with Azolla sprayed on roselle plants at 6% concentration is considered the best agricultural management; it is an effective alternative practice to increase available nutrients and yield as well as contributes to the sustainable development of medicinal crops free from harmful chemicals that negatively affect the human health.

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