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EFFECTS OF ORGANIC FERTILIZERS ON GROWTH AND YIELD OF ROSELLE (*Hibiscus sabdariffa* L.) ON BRIS SOIL

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ABSTRACT

The application of organic fertilizers on roselle has generated much interest among the researchers and farmers for the past two decades. However, little attention is given on the plantation quality in Beach Ridges Interspersed with Swales (BRIS) soil. Roselle is generally preferred because it is rich in ascorbic acid and anthocyanin that are very beneficial for health. This study determined the growth, yields and antioxidants of roselle on BRIS soils after application with different rate of organic fertilizers. Roselle was treated with five different rates of goat manure i.e. T1 (control), T2 (40 mt/ha), T3 (80 mt/ha), T4 (120 mt/ha) and T5 (160 mt/ha). Plant growth, yields and antioxidants of roselle calyxes were determined at three weeks interval for 21 weeks. Generally, organic fertilizer treated roselle showed significant increased ($p<0.05$) plant growth and plant yield. Higher rate of organic fertilizer, particularly T5 (160 mt/ha) increased the stem diameter and stem height, leaves number and leaves area as well as the biomass and number of calyx. Organic fertilizer treatments had no significant effect on ascorbic acid, carotenoids and anthocyanin production except for T3 treated plants whereas, chlorophyll content was significantly induced ($p<0.05$) in response to organic fertilizer treatments. In conclusion, application of organic fertilizers at higher rate showed positive effects on the growth, yield and antioxidant content of roselle. Therefore, organic fertilizer should be frequently applied in the farms to get the best growth and yields of the plant.

Key words: *Hibiscus sabdariffa*; organic fertilizer; BRIS soil; roselle quality; antioxidants

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) belongs to the Malvaceae family. It is recognized as tropical and sub-tropical shrub, which can be found in India, Indonesia and Malaysia (Dhar *et al*., 2015). There are three new types of roselle including red roselle, wild red roselle and yellow roselle. Three varieties such as UKMR-1, UKMR-2 and UKMR-3 had been launched and developed by Universiti Kebangsaan Malaysia. For the commercial growth, the red roselle, variety UMKL-1 also called "Terengganu" is commonly selected for planting (Naimah *et al*., 2014).

Roselle appears bright in colour and rich in nutrients such as anthocyanins, organic acids, pectin, phenolic compounds and vitamins that are important in reducing chronic diseases (Wu *et al*., 2018) as well as antispasmodic, hypotensive, antimicrobial and uterine muscle relaxation (Khalid *et al*., 2012). Roselle also could be consumed as juice, jam, beverages and foods (Rozan *et al*., 2017). Furthermore, it is cultivated for its stem as the source of pulp in paper industry (Aliyu & Tanmu, 1996). It is estimated that total market value of the roselle industry in Malaysia is RM10 to RM15 millions, approximately 65% to 80% of the value staying with the processors. Thus, there is high potential of roselle in the local market (Osman *et al*., 2011).

Roselle is now commercially planted in BRIS soil as an alternative crop to tobacco in Terengganu. BRIS soil provides a well-aerated and deep rooting zone thus, suitable for the roselle growth. However, low water holding capacity and low fertility level limits the production. Due to this, the cultivation of roselle in BRIS soil requires high organic matter to improve the soil fertility. Organic fertilizers are an important source to maintain the soil organic matter, productivity and aggregate stability. Application of organic fertilizers not only increases the soil physical condition and environmental

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quality but also provides nutrients for plant growth (Dahmardeh, 2012). Even though roselle contributes to great economic values and health benefits, only a few studies focus on effects of organic fertilizers on growth, yield and amount of antioxidants in roselle on BRIS soil. Therefore, the aim of this work is to study the effects of different rate of organic fertilizer on the growth and yield as well as to determine the production of carotenoids, ascorbic acid, anthocyanins and chlorophyll contents in roselle. The data obtained is very useful as a reference to other growers to boost the productivity of roselle.

MATERIALS AND METHODS

Plant materials

Roselle seeds of variety UKMR-2, were purchased from Department of Agriculture, Kuala Berang, Terengganu. The seeds (105 seeds) were initially sown in a germination tray containing peat moss medium. Seedlings were placed in a greenhouse at the School of Food Science and Technology, UMT. BRIS soil was taken from Department of Agriculture Commodities Center in Rhu Tapai, Terengganu. The seedlings were watered twice daily.

Fertilizers application: After one week of seedling, the processed goat manure obtained from Cosmopal Sdn. Bhd., Kuala Terengganu, was added into big polybags (20 cm x 24 cm) containing 30 kg of BRIS soil per bag. Before the transplantation of young seedlings into the polybags, the soil and goat manure were sowed to achieve homogeneity.

Transplanting: After one week of sowing, seedlings were transplanted into polybags. A total of 15 samples were used in five different treatments of goat manure. The rates of fertilizer treatment consisted of T1 (control), T2 (40 mt/ha), T3 (80 mt/ha), T4 (120 mt/ha) and T5 (160 mt/ha). Each treatment was applied in three replications. The experiment was conducted in Randomized Complete Block Design (RCBD).

Harvesting: After two months of planting, the roselle calyxes were harvested. According to FAMA (2007), the harvested calyxes were standardized at maturity index 3 where the calyxes were ripe, well developed and fresh or their seeds were visible and red-brownish in colour.

The growth measurements: Stem diameter of roselle was determined using the caliper. Stem height was determined using a measuring ruler with the aid of a thread from the soil surface to the top of the plant. The number of leaves was counted manually. Leaf area outlines were traced out on a graph paper to measure the leaf area. All the growth parameters were recorded at every three weeks interval for 21 weeks.

The yield of roselle: The fresh weight of roselle calyxes was weighed and recorded using top pan balance (SARTORIUS). For the dry weight determination, the calyxes were oven-dried for 72 hours at 60°C until constant weight were obtained. The data for fresh and dry weights were collected at 12, 15, 18 and 21 weeks of experiment.

Determination of antioxidant and chlorophyll content

Carotenoids content: This assay was carried out according to Lichtenthaler (1987). The harvested samples were cleaned and washed with tap water to remove any dirt or dust to avoid any inert debris in the assays. Calyxes (0.5g) were ground up with 3 ml of 80% (v/v) of acetone in mortar and pestle. After that, the mixture was centrifuged at 10,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 663.2 nm, 646.8 nm and 470 nm using the spectrophotometer (UV-1601 SHIMADZU) and 80% acetone was used as a blank. The carotenoids content was calculated using formula:

$$\text{Chlorophyll a, Ca (mg/L)} = 12.25A_{663.2} - 2.79A_{646.8}$$

$$\text{Chloropyll b, Cb (mg/L)} = 21.50A_{646.8} - 5.10A_{663.2}$$

$$\text{Carotenoids, Cx + c (mg/ L)} = \frac{1000A_{470} - 1.82\text{Ca} - 85.02\text{Cb}}{198}$$

Ascorbic acid: Ascorbic acid assay in calyx was conducted according to Jagota and Dani (1982). About 0.15 g of fresh sample was ground in pre-chilled mortar and pestle with 1.0 ml of 10% trichloroacetic acid (TCA) under dim light and in ice-cold conditions. The mixture was centrifuged at 5320 rpm for 10 minutes at 4°C. Supernatant (300 µl) was added into 1700 µl distilled water and 200 µl of 10% folin reagent. The mixture was gently swirled and left on bench under dim light for 10 minutes. The absorbance of the mixture was measured at 760 nm using the spectrophotometer. A standard curve was prepared using ascorbic acid at various concentrations (0-60 µg/ml). Amount of ascorbic acid in the samples were calculated based on the standard curve.

Anthocyanin content: The anthocyanin assay was conducted according to Wrolstad (1976) with a few modifications. A total of 0.15 g of calyx was blended with 3 ml of methanol. Then, the mixture was centrifuged at 3000 rpm for 5 minutes. After centrifugation, 0.5 ml of the supernatant was pipetted and diluted with 2 ml of pH 1.0 buffer. The same steps were repeated where 0.5 ml of supernatant was pipetted and diluted with 2 ml of pH 4.5 buffer. These two solutions were kept for 2 hours in a dark place. The absorbance of roselle in pH 1.0 buffer was determined at 510nm. The step was repeated for the pH 4.5 buffer solution. The absorbance was recorded

and total absorbance of anthocyanin was calculated based on formula:

Anthocyanin concentration estimation (mg/L):

$$\frac{A}{EL} \times 10^3 \times BM \times FP$$

A = absorbance of pH 1.0 – pH 4.5
E = cyanidine-3-glycoside molar absorption = 26000
L = wavelength = 1cm
BM = Molecule weight of cyanidine-3-glucoside = 445.2
FP = Dilution factor = 20

Determination of chlorophyll content

Chlorophyll content of roselle leaves was determined according to Harbone (1984). Approximately 0.15 g of samples were ground in a mortar and pestle with 6.0 ml of 80% acetone. The mixture was centrifuged at 10,000 rpm for 10 minutes. After that, the absorbance of supernatant measured at 663 nm and 645 nm using spectrophotometer. Total chlorophyll was calculated using the following formula:

$$\text{Total chlorophyll} = \frac{(20.2\ A_{645} + 8.02\ A_{663}) \times V}{\alpha \times 1000\text{ml} \times w}$$

Where,
W = leaves weight (g)
V = acetone volume (ml)
α = 1.0

Statistical analysis

Data was analyzed using analysis of variance and Statistical Package for Social Science software. Multiple means comparison was performed using Duncan Multiple Range Test at α = 0.05.

RESULTS AND DISCUSSION

Effect of organic fertilizer on growth of roselle

Stem diameter and stem height of roselle plants were generally increased throughout the experiments. Plants treated with organic fertilizer produced significantly higher ($p<0.05$) stem diameter and height especially in T5 and T4, respectively. The growth was in the range of 0.77 ± 0.07 cm to 2.53 ± 0.03 cm (stem diameter) and 16.83 ± 0.05 cm to 102.27 ± 7.07 cm (stem height) (Figures 1 & 2). The range obtained in this study was slightly higher compared to the previous study on *H. cannabinus* L. (Hazandy *et al*., 2009). They found that the stem diameter and height ranged from 0.85 cm to 1.01 cm and 79.4 cm to 89.6 cm, respectively. Similar results were also reported by El-Sherif and Sarwat (2007) in the roselle plant treated with chicken manure. Increase in plant growth particularly in higher rate of fertilizers shows that organic manures improved the soil physical conditions and promoted microbial and soil organic matter, which in turn produced organic acids. These organic acids enhanced the promotive effect of auxin, which has direct effect on plant growth (Leopold, 1974). Organic fertilizer also plays an important role to promote the root growth and enhance the nutrient absorption of plants as observed by Egharevba and Ogbomo (2007).

Figures 3 & 4 showed that the leaf numbers and leaf areas of roselle plant were significantly affected ($p<0.05$) when the plants were fertilized with organic manure. From the data obtained it could be drawn that T5 significantly increased the leaf numbers and leaf areas compared to other treatments. The increment was highest at week 9 and reduced afterwards. Previous study by Senjobil *et al*. (2010)

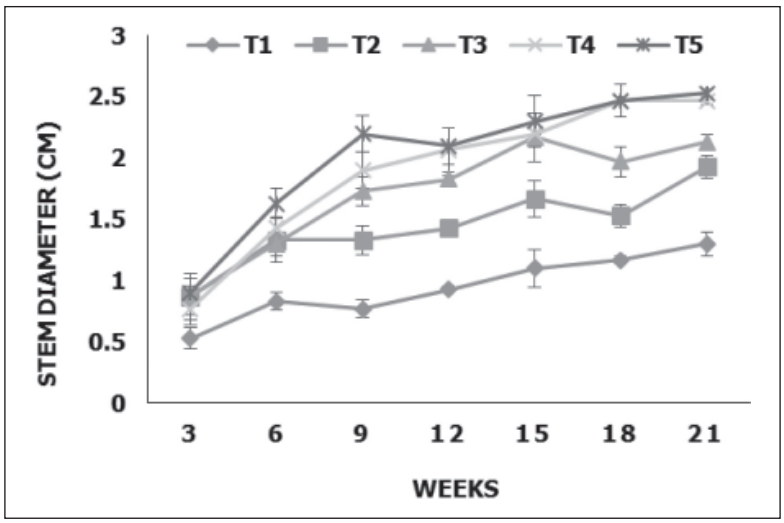

Fig. 1. Stem diameter of roselle plants (cm) treated with different organic fertilizers for 21 weeks. Data are means ± standard error (n=3). T1 (control), T2 (40 mt/ha), T3 (80 mt/ha), T4 (120 mt/ha) and T5 (160 mt/ha).

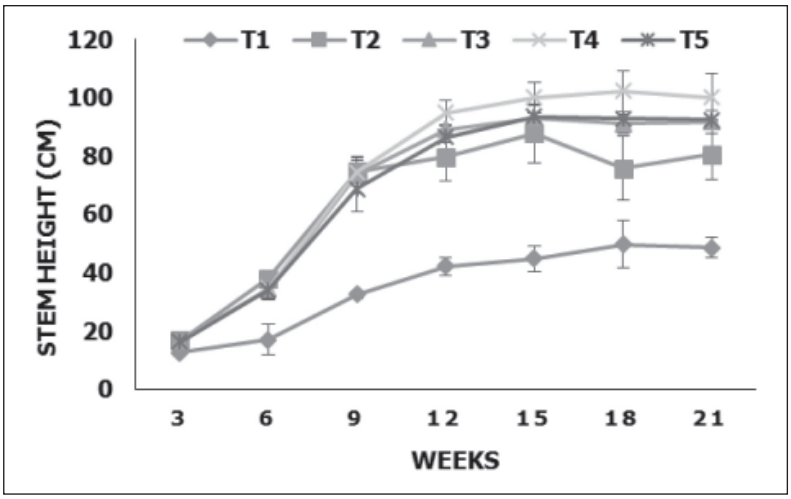

Fig. 2. Stem height of roselle plants (cm) treated with different organic fertilizers for 21 weeks. Data are means ± standard error (n=3). T1 (control), T2 (40 mt/ha), T3 (80 mt/ha), T4 (120 mt/ha) and T5 (160 mt/ha).

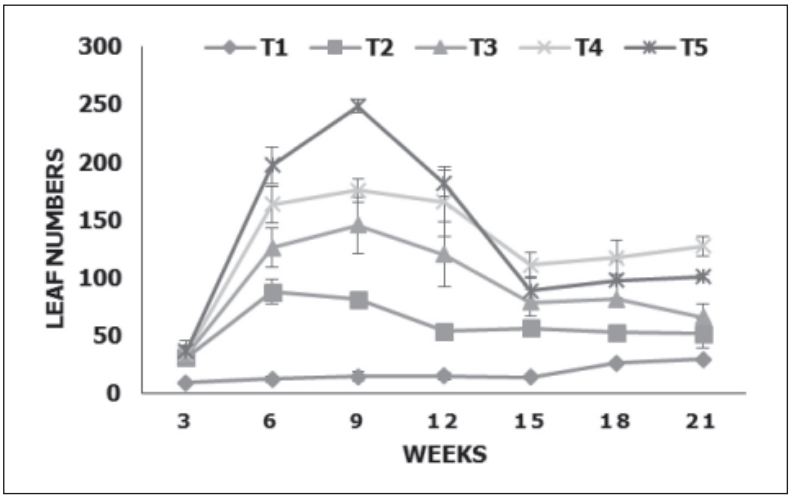

Fig. 3. Leaf number of roselle plants (cm) treated with different organic fertilizers for 21 weeks. Data are means ± standard error (n=3). T1 (control), T2 (40 mt/ha), T3 (80 mt/ha), T4 (120 mt/ha) and T5 (160 mt/ha).

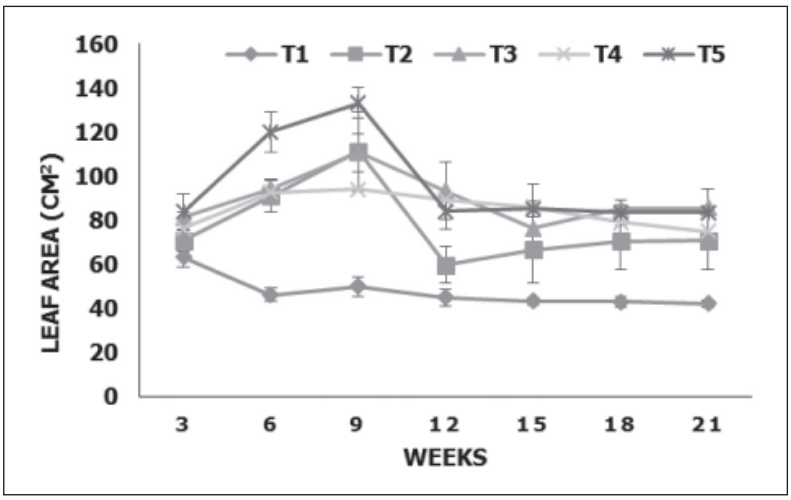

Fig. 4. Leaf area of roselle plants (cm^2) treated with different organic fertilizers for 21 weeks. Data are means ± standard error (n=3). T1 (control), T2 (40 mt/ha), T3 (80 mt/ha), T4 (120 mt/ha) and T5 (160 mt/ha).

on *Cochorus olitorius* and *Lactuca sativa* L. fertilized with chicken manure also proved that relatively higher than average leaf numbers were produced per plant (Masarirambi *et al*., 2010). Decreasing trend at latter stages might be due to the reduction of soil nutrients, soil water potential and leaves senescence. Soil drying decreased leaf growth thereby reducing leaf water status and accumulation of organic solutes which in turn inhibited the incorporation of substrate molecules to grow new cell, hence the leaf numbers also decreased. At the later stage of plant growth, leaves became senesced and the low pressure of turgidity of plants influenced the cell enlargement and affected plant growth (Reddy *et al*., 2003).

Effect of organic fertilizer on yield of roselle

Application of organic fertilizers also increased the fresh and dry weights of roselle calyxes especially at week 15 (Figures 5A & B). Results revealed that roselle treated with higher rate of goat manure (T4) produced highest fresh weight of

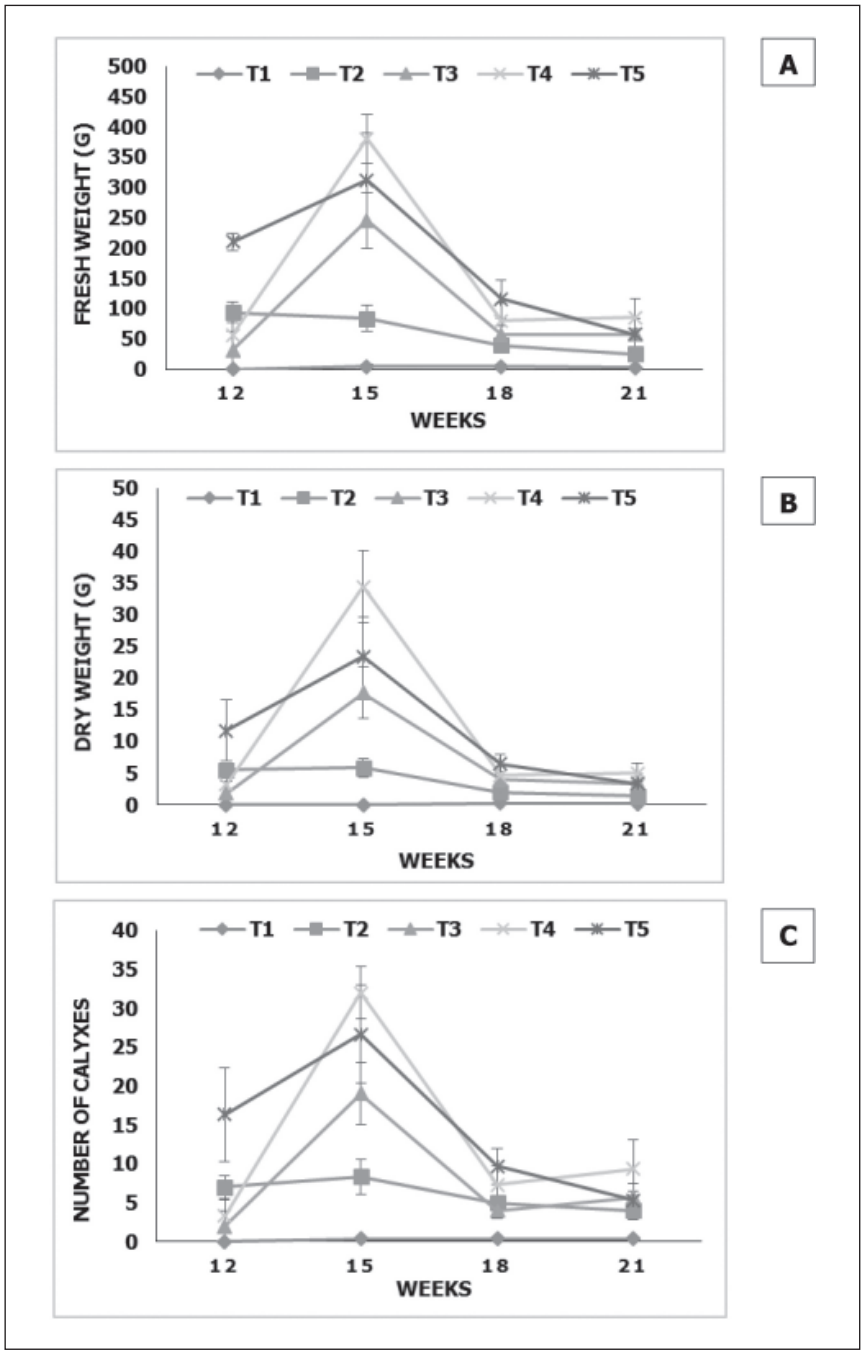

Fig. 5. Fresh (A), dry (B) weights (g) and number of roselle calyxes (C) treated with different organic fertilizers for 21 weeks. Data are means ± standard error (n=3). T1 (control), T2 (40 mt/ha), T3 (80 mt/ha), T4 (120 mt/ha) and T5 (160 mt/ha).

Table 1. Effect of organic fertilizer application on the production of carotenoids, ascorbic acid, anthocyanin and chlorophyll contents of roselle at 21 weeks of treatments

Treatment (mt/ha)	Carotenoid (mg/L)	Ascorbic acid (mg/g Fwt)	Anthocyanin (mg/L)	Chlorophyll (mg/g Fwt)
T1 (control)	4.02±0.95^{a}	28.58±2.00^{a}	83.60±12.00^{a}	0.42±0.10^{a}
T2 (40)	2.19±0.39^{a}	25.05±2.00^{a}	121.09±7.59^{ab}	0.64±0.05^{b}
T3 (80)	3.56±0.49^{a}	27.22±2.00^{a}	141.00±26.27^{b}	0.61±0.02^{b}
T4 (120)	2.16±0.25^{a}	30.04±3.54^{a}	105.41±2.12^{ab}	0.63±0.41^{b}
T5 (160)	2.86±0.51^{a}	31.28±3.79^{a}	116.84±10.31^{ab}	0.59±0.02^{b}

Data are means ± standard error (n=3). Different letters in a column represent significantly different at $p<0.05$.

380.15 ± 40.81 g compared to other treatments and control. The more application of these fertilizers accumulated additional solutes in the soil, thus improved the vegetative growth and dry matter yield (Gendy *et al*., 2012). Study by Sharma *et al*. (2003) also showed that organic manures have beneficial effects on the yield of plants because of the ability of plant to accumulate materials into the storage and economic parts of plant.

The present results also showed higher number of calyxes in organic fertilizer treated roselle compared to control (Figure 5C). El-Sherif and Sarwat (2007) also found out that the number of roselle's fruit increased significantly when the organic waste compost was incorporated into sandy soil. It can be suggested that application of organic fertilizers led to the enhancement of soil physical criteria including better aeration, better water holding capacity, better nutrient availability and good balance between nutrients in the soil solution and improvement of nutrient exchange between the soil. Slow release of nutrients from the organic fertilizers during growth period and hence low leaching of the nutrients could also be other criteria, which improved vegetative growth and flower induction that are related to sepal's weight and the yield index (Gendy *et al*., 2012).

Effect of organic fertilizer on antioxidants and chlorophyll content of roselle

Genetics, environment and post-harvest practices might influence the nutritional composition of vegetables grown organically as observed by Zahradnik and Petríkova (2007). There was no significant difference ($p>0.05$) in the carotenoids and ascorbic acid content among the treatments and control (Table 1). T3 contained the highest anthocyanin of 141.00 ± 26.27 mg/L compared to other treatments. Similarly, Tsai and Huang (2004) also found that roselle treated with organic compost promote the total anthocyanin content. In this study, higher rate of organic fertilizers produced lower anthocyanin content. The lowest anthocyanin content was observed in T4 treated plants. Organic fertilizer increased the availability and uptake of nutrients, which enhanced the higher photosynthetic activity and in turn, corresponded to the higher amounts of anthocyanin. Anthocyanin content slightly varied among the different treatments of goat manure, this might be due to unequal light intensity absorbed by the plants, thus affecting the accumulation of anthocyanin. Besides the light intensity, temperature are also another main environmental factors important for red pigments development in fruits.

The chlorophyll content of roselle in the present study was in the range of 0.42 ± 0.10 mg/g Fwt to 0.64 ± 0.05 mg/g Fwt. The chlorophyll content was positively affected as a result of using organic fertilizers. Increase in the chlorophyll content might be related to the incensement of photosynthetic rate in plants as reported by Karanatsidis and Berova (2009). The low chlorophyll content of roselle possibly might be due to the water deficiency, which inhibited plant growth (Ashraf & Harris, 2005).

CONCLUSION

Organic fertilizers showed positive effects on the growth, yields and anthocyanin contents of roselle. Application of organic fertilizers at higher rate (160 mt/ha) increased the growth and yield of roselle plant. However, addition of organic fertilizers have no significant effect on the antioxidants studied.

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