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Vol. 7(4), pp. 45-52, April, 2016
DOI 10.5897/JSSEM2015.0552
Articles Number: C85FAF457958
ISSN 2141-2391

http://www.academicjournals.org/JSSEM

Journal of Soil Science and Environmental Management

*Full Length Research Paper*

# Effect of silicon application on roselle (*Hibiscus sabdariffa* L.) grown in a Vertisol in Egypt

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Received 20 January, 2016; Accepted 22 February, 2016

**Roselle (*Hibiscus sabdariffa* L.) is an important tropical and subtropical crop, because of its multi uses in the medicinal purposes as well as food industries. A plot experiment was conducted in a Vertisol in Egypt over two sequential seasons (2013 and 2014) to assess the effect of silicon (Si) fertilization on roselle growth and yield. Specific growth characteristics measured were: plant heights, branching, and leaves; biomass and calyces yield; and concentrations of anthocyanin, total soluble solids (TSS), carbohydrates, N, P, and K in the calyces. The experiment compared five rates of Si fertilization (0.00, 1.75, 3.50, 5.25, and 7.00 kg Si ha$^{-1}$). One-third of each rate was applied as a foliar spray at 45, 60, and 75 days after sowing, respectively. Results showed an increase in plant height, number of branches and leaves to Si fertilization rates. Similarly, anthocyanin and TSS concentration increased with increasing Si rates. Anthocyanin concentration significantly increased by 16.3% as the applied Si rate increased from 0.00 to 5.25 kg Si ha$^{-1}$. However, carbohydrate content was not affected by the applied Si rates. Nutrients (N, P, and K) concentrations, in the calyces extract, increased with increasing the applied Si rate. Biomass and calyces yield increased by 23 and 33%, respectively, as the applied Si rate increased from 0.00 to 5.25 kg Si ha$^{-1}$. The highest values of all of the measured properties were observed under the Si rate of 5.25 kg Si ha$^{-1}$, and the lowest values were obtained from the control treatment (0.00 kg Si ha$^{-1}$).**

**Key words:** Calyces, anthocyanin, hibiscus, silicon.

## INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is a tropical and subtropical annual herbaceous shrub belonging to the family Malvaceae. In North Africa, especially in Egypt, it is known as Karkadeh. Roselle could be native to Africa and is widely cultivated throughout many countries such as India, Sudan, Egypt, and Mexico (Tindall, 1983). It could be planted in the entire field as the main crop, intercropped with other crops, or planted along the margins of the field. Roselle could be grown for medicinal, fiber, and beverage purposes (Igarashi et al., 1989; Tsai et al., 2002).

Roselle calyces are rich in the anthocyanin pigments,

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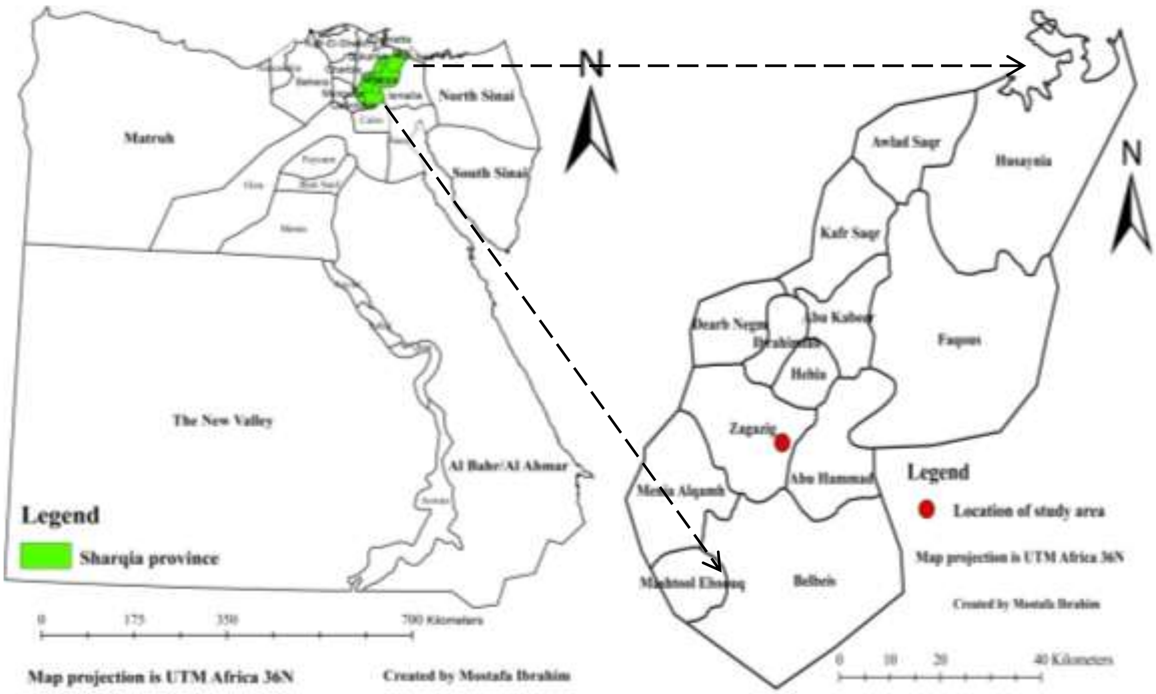


**Figure 1.** Study area location in Zagazig County, Sharqia province, Egypt.

which in turn are responsible for creating the red color and flavor in roselle. In the food industries, anthocyanin pigments were suggested to be used as food colorants and emulsifiers in the carbonated drinks (Duangmal et al., 2004). Anthocyanins have been successfully used in medicinal purposes, e.g., as a treatment of inflammatory, cancer, and liver diseases (Kong et al., 2003). Unfortunately, few literatures were published about roselle in terms of the influence of different nutrients on its biomass and calyces yield. Spontaneously, no work has been published about applying Si as a fertilizer to roselle.

Silicon (Si) is ubiquitous in nature and is considered the second most abundant element after oxygen comprising ~28% by weight of the earth's crust (Wedepohl, 1995). Plants take up Si, especially at alkaline pH, in the soluble form of mono-silicic acid ($Si(OH)_4$) or the ionic form $Si(OH)_3O^-$, which occurs in low concentrations in the soil solution (Currie and Perry, 2007). Silicon concentration in most of the soils may range from <1 to 200 mg Si $kg^{-1}$ soil (Sommer et al., 2006; Ibrahim and Lal, 2014). Although concentration of the plant available form of Si (mono-silicic acid) in soils is low, its concentration in plants was reported to range from 1 to 100 g Si $kg^{-1}$ plant dry matter, which is equal to or even larger than the amounts of many macro-nutrients in plants (Epstein, 1994). Although Si has not been considered an essential element for higher plants, it was found to be beneficial to several plants. For example, applying Si to rice, sugarcane, wheat, and barley increased their biomass and crop yield (Makabe et al., 2009; Meyer and Keeping, 2001; Ahmed, 2014; Liang, 1999). In the past several decades, Si was not considered an essential element for higher plants. However, Epstein and Bloom (2005) defined new criteria of the essential elements for higher plants upon which Si could be considered an essential element. Applying silicon to roselle has been hypothesized to increase the biomass and calyces yield by enhancing the leaves erection, strengthening roselle roots, and increasing the uptake of other nutrients (e.g., N, P, and K). The aim of this work was to assess the influence of Si as a fertilizer on roselle in terms of its growth characteristics, content of anthocyanins, TSS, carbohydrate, nutrients (N, P, and K), biomass, and calyces yield.

## MATERIALS AND METHODS

### Location, design of the experiment, and soil analysis

The experiment was conducted in two consecutive seasons of 2013 and 2014 at Zagazig University experimental farm at Ghazala Village, Zagazig, Sharqia, Egypt (30° 34' 07" N, 31° 34' 33" E) (Figure 1). The monthly average temperature ranges from 9°C in January to 34°C in July and August and the mean annual precipitation is 51 mm (World Meteorological Organization Staff, 2015). The experiment consisted of five silicon treatments distributed in a completely randomized block design. Each treatment was replicated four times (four plots). Each plot had a width of 2 m and a length of 2.4 m. Four rows were manually constructed within each plot 60 cm apart. Within each row, plants were 50 cm apart. Roselle seeds were obtained from the

**Table 1.** Some selected soil properties of the experiment area.

| Year | pH (1:1) | EC (1:2.5) (dSm$^{-1}$) | OM (g kg$^{-1}$)† | Particle size analysis | | |
|---|---|---|---|---|---|---|
| | | | | Sand (%) | Silt (%) | Clay (%) |
| 2013 | 7.86 | 0.95 | 5.2 | 19.71 | 31.93 | 48.36 |
| 2014 | 7.79 | 0.98 | 5.4 | 18.24 | 33.12 | 48.64 |

†OM: Organic matter.

Agricultural Research Center, Giza, Egypt. In order to insure that nitrogen and phosphorus were not limiting and following the recommended rates by other authors (Haruna et al., 2009), 120 kg N ha$^{-1}$ as ammonium nitrate and 33.2 kg P ha$^{-1}$ as ordinary super phosphate were applied over each growing season. Phosphorus was added during the soil preparation before sowing and nitrogen was added in three equal amounts of 40 kg N ha$^{-1}$ at 40, 70, and 100 days after sowing. Weed control was carried out manually throughout the growing season.

The seeds were sown in the first week of June in the two growing seasons. Five rates of Si (0.00, 1.75, 3.50, 5.25, and 7.00 kg Si ha$^{-1}$) were applied three times as a foliar spray in three equal amounts (each was one third of the aforementioned Si rates) at 45, 60, and 75 days after sowing. A high concentrated solution of Si was prepared by dissolving a specific weight of K-silicate ($K_2SiO_3$) powder (AgSil 16H, 52.8% $SiO_2$, PQ Silicates Ltd., Taipei, Taiwan) in distilled water. From the concentrated solution, dilutions were made to obtain the used concentrations (treatments). Potassium concentration was adjusted in all of the treatments to become constant using KCl. Distilled water was sprayed to represent the 0.00 kg Si ha$^{-1}$ application.

The experiment was conducted in a clayey soil, which classifies as a Vertisol using Keys to Soil Taxonomy (Soil Survey Staff, 2015). Before planting, four soil samples were manually collected at a depth of 30 cm using a shovel from the experimental area. Soil samples were air dried, crushed, and sieved to pass a 2 mm screen to separate the fine fraction from the coarse fraction. All of the soil analyses (Table 1) were conducted on the fine fraction (<2 mm in diameter). Soil texture was determined using the pipette method (Pansu and Gautheyrou, 2006). Salinity concentration was measured using a 1:2.5 soil: water suspension using an EC meter (Thermo Scientific, Beverly, MA, USA). Soil pH was determined in a 1:1 (soil: water) suspension using an Orion pH meter (Thermo Scientific, Beverly, MA, USA). Soil organic matter was determined using the loss on ignition (LOI) method (Davies, 1974). Soil inorganic N was extracted by a 2 mol L$^{-1}$ KCl solution and measured using the micro-kjeldahl apparatus (Keeney and Nelson, 1982). Soil inorganic P was extracted by 0.5 mol L$^{-1}$ $NaHCO_3$ adjusted at pH 8.5. Phosphorus in the extraction was measured colorimetrically at a wavelength of 750 nm using a spectrophotometer (Milton Roy Spectronic 401, Ivyland, PA, USA) (Watanabe and Olsen, 1965). Soil K was extracted using a 1 mol L$^{-1}$ $NH_4OAC$ solution (Jackson, 2005) and measured using an atomic absorption spectrometer (PerkinElmer Instruments, Waltham, MA, USA).

### Field sampling, biomass, and calyces yield determination

Shortly before harvest, some growth characteristics (e.g., plant height, number of branches/plant, number of leaves/plant) were determined. This information was recorded 130 day after sowing. Each characteristic was determined in three randomly collected plants in each plot and the average of the three obtained values was used for the later data analysis. For example, plant height was measured from the base of the stem to the apex of the last leaf, the number of branches was determined by counting the primary productive branches, and the number of leaves was determined by counting all of them produced in each plant. Roselle plants were harvested 160 days after sowing. To determine the biomass and calyces yield, plants of a central row (4 plants/row) from each plot were harvested at 5 cm above the ground. Roselle plants were harvested on 21 November, 2013 for the first season and on 15 November, 2014 for the second season. For the biomass determination, the entire sampled plants (stems, leaves, and fruits) were weighed in the field and small parts of the stem, leaves, and fruits were transferred in a paper bag to the laboratory to determine the dry weight. Fruits of the four plants were separated in the field, transferred to the laboratory in paper bags, and calyces were separated from the seeds capsules using a simple hand tool. In the laboratory, all of the shoots (stem and leaves) samples and calyces samples were air dried under the room temperature for 4 days and then transferred to an oven adjusted at 65°C for 3 days until the weight became constant. After drying shoots and calyces samples in the oven, biomass and calyces yield were determined on a dry weight basis.

### Plant analyses

Dried calyces materials were ground**,** using a Wiley mill (Thomas-Wiley Co., Philadelphia, PA, USA) to pass a 2-mm screen, and were reground to uniformity and pass through a 1-mm screen using a UDY-Cyclone impact mill (UDY Corporation, Fort Collins, CO, USA). All of the ground subsamples were stored in polyethylene bottles for further analyses. To determine anthocyanin, in a 250 ml beaker, 1 g of the dried ground calyces was macerated in 30 ml of ethanol (95%) and 1.5 mol L$^{-1}$ HCl mixture (85 ethanol:15 HCl, v/v) for 24 h at 4°C. Afterwards, all of the contents were filtered in a 100 ml volumetric flask using a filter paper (Whatman No. 1). The residue on the filter paper was re-extracted two more times and filtered in the 100 ml volumetric flask until the filtered solution became colorless (Abou-Arab et al., 2011). Anthocyanin in the extract was measured colorimetrically at a 535 nm wavelength using a spectrophotometer (Milton Roy Spectronic 401, Ivyland, PA, USA) following the method documented by Du and Francis (1973). Total soluble solids content was determined using an Atago refractometer (Atago Co. Ltd. Tokyo, Japan). Total carbohydrate content was determined colorimetrically at 490 nm wavelength using a spectrophotometer (Milton Roy Spectronic 401, Ivyland, PA, USA) (Dubois et al., 1956).

To determine N, P, and K, on a hot plate, 0.3 g of dried and ground calyces materials was digested with 4 ml concentrated $H_2SO_4$ and 1 ml of concentrated $HClO_4$. The digested materials were quantitatively transferred to a 100 ml volumetric flask using distilled water. N was determined using the distillation method using a micro-kjeldahl apparatus (Matsoukis et al., 2007). Phosphorus was determined colorimetrically at 750 nm wavelength using a spectrophotometer (Milton Roy Spectronic 401, Ivyland, PA, USA) (Watanabe and Olsen, 1965). Potassium was measured using an

**Table 2.** Some vegetative growth characteristics of roselle plants fertilized with different rates of Si during the two seasons (2013 and 2014). Values represent means of 4 replicates, ($P \leq 0.05$). Within each column, similar letters indicate no significant difference and different letters indicate significant difference.

| Silicon rate (kg $h^{-1}$) | First season (2013) | | | Second season (2014) | | |
|---|---|---|---|---|---|---|
| | Plant height (cm) | Number of branches | Number of leaves | Plant height (cm) | Number of branches | Number of leaves |
| 0.00 | 153.3$^{c}$ | 11.3$^{b}$ | 184.1$^{d}$ | 152.7$^{c}$ | 10.6$^{b}$ | 182.3$^{d}$ |
| 1.75 | 156.7$^{c}$ | 11.7$^{b}$ | 216.2$^{c}$ | 153.1$^{c}$ | 10.9$^{b}$ | 211.5$^{c}$ |
| 3.50 | 165.1$^{b}$ | 13.0$^{ab}$ | 253.7$^{b}$ | 164.8$^{b}$ | 12.7$^{ab}$ | 248.3$^{b}$ |
| 5.25 | 182.3$^{a}$ | 13.7$^{a}$ | 291.1$^{a}$ | 178.9$^{a}$ | 13.5$^{a}$ | 283.6$^{e}$ |
| 7.00 | 169.7$^{b}$ | 12.7$^{ab}$ | 210.7$^{c}$ | 166.3$^{b}$ | 12.5$^{ab}$ | 205.2$^{c}$ |

**Table 3.** Some chemicals constituents of roselle calyces fertilized with different rates of Si during the two seasons (2013 and 2014). Values represent means of 4 replicates, ($P \leq 0.05$). Within each column, similar letters indicate no significant difference and different letters indicate significant difference.

| Silicon rate (kg $h^{-1}$) | First season (2013) | | | Second season (2014) | | |
|---|---|---|---|---|---|---|
| | Anthocyanin (mg $g^{-1}$) | TSS[†] (g $kg^{-1}$) | Carbohydrate (g $kg^{-1}$) | Anthocyanin (mg $g^{-1}$) | TSS (g $kg^{-1}$) | Carbohydrate (g $kg^{-1}$) |
| 0.00 | 10.4$^{b}$ | 75.1$^{b}$ | 172$^{a}$ | 9.3$^{b}$ | 74.5$^{b}$ | 168$^{a}$ |
| 1.75 | 10.7$^{b}$ | 76.4$^{ab}$ | 158$^{a}$ | 9.8$^{b}$ | 75.3$^{b}$ | 153$^{a}$ |
| 3.50 | 11.1$^{b}$ | 77.5$^{a}$ | 163$^{a}$ | 10.7$^{b}$ | 76.8$^{a}$ | 159$^{a}$ |
| 5.25 | 12.1$^{a}$ | 78.3$^{a}$ | 165$^{a}$ | 11.9$^{a}$ | 77.6$^{a}$ | 162$^{a}$ |
| 7.00 | 11.7$^{a}$ | 76.8$^{ab}$ | 165$^{a}$ | 11.5$^{a}$ | 74.1$^{b}$ | 161$^{a}$ |

[†]TSS: Total soluble solids.

atomic absorption spectrometer (PerkinElmer Instruments, Waltham, MA, USA). All statistical analyses were carried out using SAS version 9.3 software (SAS Institute, 2011). The ANOVA test was carried out using PROC GLM of SAS to compare the means of the different treatments. Significant differences were determined at a 0.05 level.

## RESULTS

Application of Si to roselle during the first season (2013) and the second season (2014) improved its growth characteristics, biomass, calyces yield, and anthocyanin, but carbohydrate content was not affected. Plant height, in the two seasons, was significantly affected by the rate of applied Si. For example, in the first season, plant height significantly increased from 153.3 to 182.3 cm as the applied Si rate increased from 0.00 to 5.25 kg Si $ha^{-1}$ (Table 2). Interestingly, the number of the productive branches was at best only slightly influenced by Si fertilization. That is, there were no significant differences among the number of branches in most of the applied Si rates (Table 2). During the two seasons, number of the leaves significantly increased as the applied Si rate increased (Table 2). For example, it proportionally increased with increasing Si rate until reaching the highest number at Si rate of 5.25 kg Si $ha^{-1}$, but it decreased after that at the Si rate of 7.00 kg Si $ha^{-1}$ (Table 2).

In the experiment and during the first and second seasons (2013 and 2014), anthocyanin content was slightly affected by the lower applied rates of Si (1.75 and 3.50 kg Si $ha^{-1}$), but it significantly increased as the applied Si rate reached 5.25 and 7.00 kg Si $ha^{-1}$ (Table 3). Compared with its concentration in the control Si treatment (0.00 kg Si $ha^{-1}$), TSS was significantly higher under the applied Si rates (3.50 and 5.25 kg Si $ha^{-1}$); however, TSS was not significantly different from the applied Si rates (1.75 and 7.00 kg Si $ha^{-1}$), in the two seasons (Table 3). The highest TSS value was observed under the Si rate of 5.25 kg Si $ha^{-1}$ and the lowest value was found under the control treatment (0.0 kg Si $h^{-1}$). Within the two seasons, carbohydrate content was not affected by the different rates of Si (Table 3).

In the two seasons of 2013 and 2014, N, and K concentrations in the calyces were significantly higher under all of the applied Si rates (1.75, 3.50, 5.25, and 7.0 kg Si $ha^{-1}$) compared with those under the control treatment (0.0 kg Si $ha^{-1}$) (Table 4). Phosphorus concentration was not significantly different between the control treatment and the applied Si rate of 1.75 kg Si $ha^{-1}$. However, P concentration under the applied Si rates of 3.50, 5.25, and 7.00 kg Si $ha^{-1}$ was significantly different compared with the control treatment (Table 4). The highest concentrations of N, P, and K were found under

**Table 4.** Concentrations of N, P, and K in calyces of roselle fertilized with different rates of Si during the first and second seasons (2013 and 2014). Values represent means of 4 replicates, ($P \leq 0.05$). Within each column, similar letters indicate no significant difference and different letters indicate significant difference.

| Silicon rate (kg $h^{-1}$) | First season (2013) | | | Second season (2014) | | |
|---|---|---|---|---|---|---|
| | N | P | K | N | P | K |
| | | (g $kg^{-1}$) | | | (g $kg^{-1}$) | |
| 0.00 | $23.1^{d}$ | $3.2^{b}$ | $25.4^{d}$ | $22.3^{d}$ | $2.9^{b}$ | $24.8^{d}$ |
| 1.75 | $25.8^{c}$ | $3.5^{b}$ | $26.4^{c}$ | $24.8^{c}$ | $3.1^{b}$ | $25.9^{c}$ |
| 3.50 | $27.2b^{c}$ | $3.7^{a}$ | $26.7^{b}$ | $26.3^{bc}$ | $3.4^{a}$ | $26.1^{b}$ |
| 5.25 | $29.7^{a}$ | $3.8^{a}$ | $27.2^{a}$ | $28.8^{a}$ | $3.6^{a}$ | $26.7^{a}$ |
| 7.00 | $28.6^{ab}$ | $3.7^{a}$ | $26.8^{ab}$ | $27.6^{ab}$ | $3.5^{a}$ | $26.4a^{b}$ |

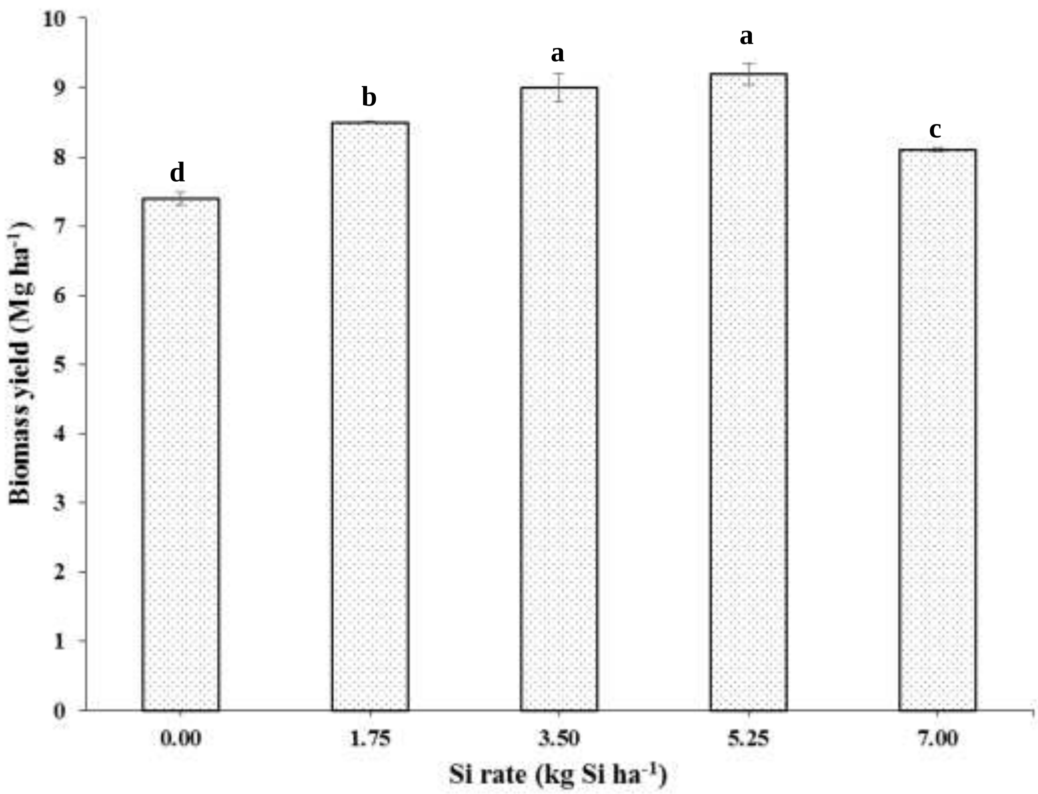


**Figure 2.** Biomass yield of roselle under different Si rates during the first season (2013). Biomass values represent the mean values of 4 replicates ($P \leq 0.05$). Error bars represent standard error.

the Si rate of 5.25 kg Si $ha^{-1}$. The lowest concentrations of N, P, and K were found under the control treatment (0.00 kg Si $ha^{-1}$).

Roselle biomass significantly increased as the applied Si rate increased within the two seasons (Figures 2 and 3). For example, within the first season (2013), biomass yield increased by 23% when the applied Si rate increased from 0.00 to 5.25 kg Si $ha^{-1}$ (Fig. 2). Similarly, Calyces yield significantly increased when the applied Si rate increased (Figures 4 and 5). For example, within the first season (2013) calyces yield increased by 33% as the applied Si rate increased from 0.0 to 5.25 kg Si $ha^{-1}$ (Figure 4).

## DISCUSSION

In general, Si impacts on plant growth depend on plant type, plant cultivar, and growing conditions. Applying Si to roselle increased plant height which could be attributed to the role of Si in elongating and strengthening plant roots resulting in increasing the ability to take up higher

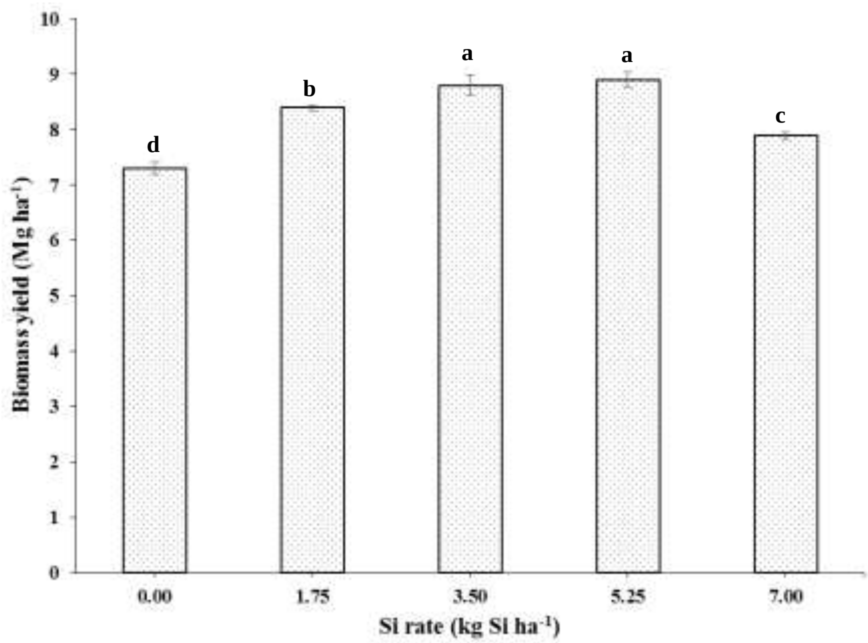


**Figure 3.** Biomass yield of roselle under different Si rates during the second season (2014). Biomass values represent the mean values of 4 replicates (P ≤ 0.05). Error bars represent standard error.

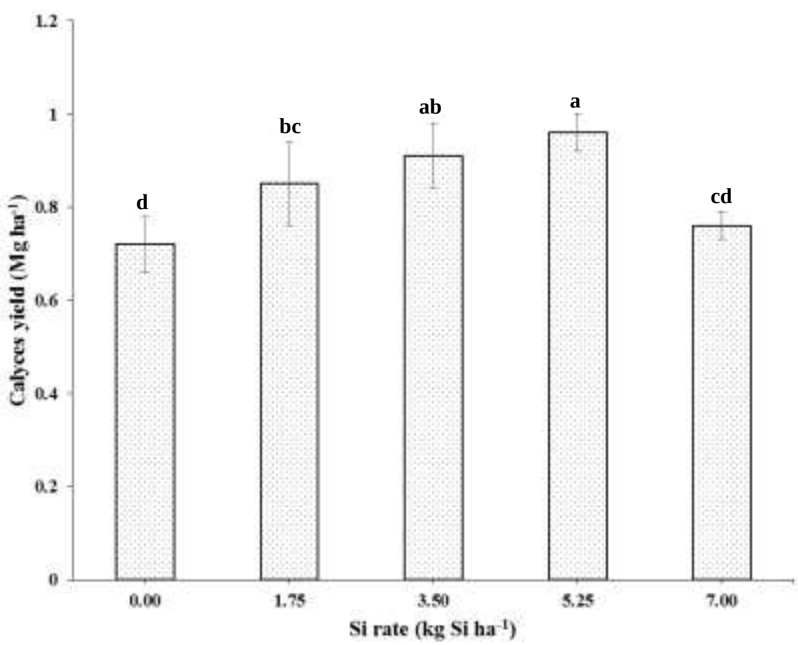


**Figure 4.** Calyces yield of roselle under different Si rates during the first season (2013). Calyces yield values represent the mean values of 4 replicates (P ≤ 0.05). Error bars represent standard error.

amounts of nutrients from the soil solution (Table 4) (Ma and Yamaji, 2006). The highest number of the productive branches was observed under the applied Si rate of 5.25 kg Si $ha^{-1}$ and this was significantly higher than that found under the control treatment – Si rate of 0.00 kg Si $ha^{-1}$ (Table 2). The number of branches and the number of

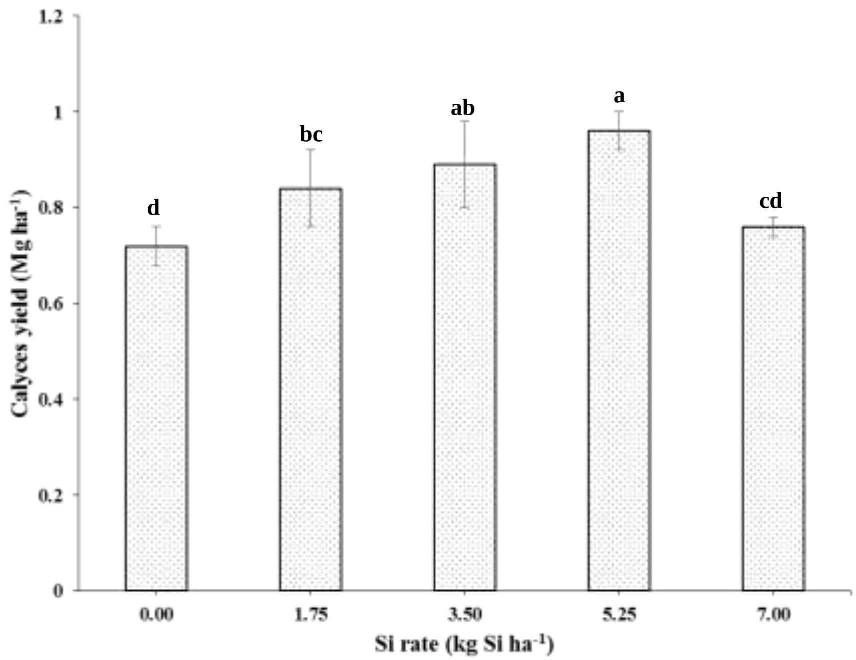


**Figure 5.** Calyces yield of roselle under different Si rates during the second season (2014). Calyces yield values represent the mean values of 4 replicates ($P \leq 0.05$). Error bars represent standard error.

leaves per plant under the applied Si rates were higher than those produced under N applications in Guinea (Giginyu and Fagbayide, 2009). Anthocyanin content was influenced by the rates of applied Si. For example, in the first season, anthocyanin concentration increased by 16.3% as Si rate increased from 0.00 to 5.25 kg Si ha$^{-1}$.

Applying Si to plants increased the uptake of other nutrients such as N, P, K, and Ca. For example, when Si was applied to wheat and cowpea, concentrations of N, K, and Ca in plant tissues increased compared with those in plants received no Si (Mali and Aery, 2008a, 2008b). Similarly, P concentration in some grasses was found to be higher under Si applications compared with P concentration in grasses received no Si (Eneji et al., 2008). Nutrients (N, P, and K) concentrations in the calyces increased as the applied Si fertilization rate increased. This increase in nutrients concentrations could be due to the applied Si that enhanced the elongation and rigidity of plant roots resulting in increasing the ability of roots to penetrate the soil and take up higher amounts of nutrients from the soil solution (Ma and Yamaji, 2006). The increase in N concentration in calyces implies an increase in the protein content of the calyces, that is, quality of the calyces increased.

The increase in biomass could be ascribed to the role of Si in enhancing leaves erection leading to improving light interception and eventually resulting in improving photosynthetic activity (Ma and Takahashi, 2002). Additionally, this increase in biomass could be due to the increase of nutrients uptake as Si enhances root growth (Ma and Yamaji, 2006).

## Conclusion

In conclusion, applying Si to roselle improved its growth characteristics. Anthocyanin concentration increased by 16.3% as the applied Si rate increased from 0.00 to 5.25 kg Si ha$^{-1}$. Similarly, TSS concentration increased with increasing the applied Si rate. Conversely, the concentration of carbohydrate in the calyces was not influenced by the applied Si rates. Nutrients (N, P, and K) concentrations increased with increasing the applied Si rate. Similarly, Biomass and calyces yield increased by 23 and 33%, respectively as the applied Si rate increased from 0.00 to 5.25 kg Si ha$^{-1}$. Overall, the highest values of all of the measured properties were obtained from the Si rate of 5.25 kg Si ha$^{-1}$, and the lowest values were observed under the control treatment (0.00 kg Si ha$^{-1}$).

## Conflict of Interests

The authors have not declared any conflict of interests.

### REFERENCES

Abou-Arab AA, Abu-Salem FM, Abou-Arab EA (2011). Physico-chemical properties of natural pigments (anthocyanin) extracted from roselle calyces (*Hibiscus subdariffa*). J. Am. Sci. 7:445-456.

Ahmad B (2014). Interactive effects of silicon and potassium nitrate in improving salt tolerance of wheat. J. Integr. Agric. 13:1889-1899.
Currie HA, Perry CC (2007). Silica in plants: Biological, biochemical and chemical studies. Ann. Bot. 100:1383-1389.
Davies BE (1974). Loss-on-ignition as an estimate of soil organic matter. Soil Sci. Soc. Am. J. 38:150-151.
Du CT, Francis FJ (1973). Anthocyanins of roselle (*Hibiscus sabdariffa,* L.). J. Food Sci. 38:810-812.
Duangmal K, Saicheua B, Sueeprasan S (2004). Roselle anthocyanins as a natural food colorant and improvement of its color stability. Proceedings of the AIC. pp. 155-158.
Dubois M, Gilles KA, Robers JH, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
Eneji AE, Inanaga S, Muranaka S, Li J, Hattori T, An P, Tsuji W (2008). Growth and nutrient use in four grasses under drought stress as mediated by silicon fertilizers. J. Plant Nutr. 31:355-365.
Epstein E (1994). The anomaly of silicon in plant biology. Proc. Natl. Acad. Sci. 91:11-17.
Epstein E, Bloom AJ (2005). Mineral nutrition of plants: principles and perspectives, 2nd ed. Sunderland, MA, USA. P 48.
Giginyu BM, Fagbayide JA (2009). Effect of Nitrogen fertilizer on the growth and calyces yield of two cultivars of roselle in Northern guinea savanna. Middle-East. J. Sci. Res. 4:66-71.
Haruna IM, Maunde SM, Yahuza S (2009). Growth and calyces yield of roselle (*Hibiscus sabdariffa L*.) as affected by poultry manure and nitrogen fertilizer rates in the southern guinea savanna of Nigeria. Can. J. Pure Appl. Sci. 5:1345-1348.
Ibrahim MA, Lal R (2014). Soil Carbon and silicon pools across an un-drained toposequence in central Ohio. Catena 120:57-63.
Igarashi K, Takanashi K, Makino M, Yasui T (1989). Antioxidative activity of major anthocyanin isolated from wild grapes (*Vitis coignetiae*). J. Jpn. Soc. Food Sci. Technol. 36:852-856
Jackson ML (2005). Soil chemical analysis: advanced course. UW-Madison Libraries Parallel Press, Madison, WI, USA pp. pp. 29-83.
Keeney DR, Nelson DW (1982). Nitrogen-inorganic forms, in: Page AL. (Ed.). Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, 2nd edition. ASA and SSSA, Madison, WI. pp. 643-698.
Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003). Analysis and biological activities of anthocyanins. Phytochemistry 64:923-933.
Liang Y (1999). Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil 209:217-224.
Ma JF, Takahashi E (2002). Soil, Fertilizer, and Plant Silicon Research in Japan. Elsevier Science, Amsterdam, the Netherlands. pp. 107-180.
Ma JF, Yamaji N (2006). Silicon uptake and accumulation in higher plants. Trends Plant Sci. 11:392-397.
Makabe S, Kakuda K, Sasaki Y, Ando T, Fujii H, Ando H (2009). Relationship between mineral composition or soil texture and available silicon in alluvial paddy soils on the Shounai Plain, Japan. Soil Sci. Plant Nutr. 55:300-308.
Mali M, Aery NC (2008a). Influence of silicon on growth, relative water contents and uptake of silicon, calcium and potassium in wheat grown in nutrient solution. J. Plant Nutr. 31:1867-1876.
Mali M, Aery NC (2008b). Silicon effects on nodule growth, dry matter production, and mineral nutrition of cowpea (*Vigna unguiculata*). J. Plant Nutr. Soil Sci. 171:835-840.
Matsoukis A, Gasparatos D, Chronopoulou-Sereli A (2007). Impact of shading and chlormequat chloride on lantana specific leaf area and mineral content. Commun. Soil Sci. Plant Anal. 38:2323-2331.
Pansu M, Gautheyrou J (2006). Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods. Springer, Berlin, Germany. pp. 15-42.
Soil Survey Staff (2014). Keys to Soil Taxonomy. 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.
Sommer M, Kaczorek D, Kuzyakov Y, Breuer J (2006). Silicon pools and fluxes in soils and landscapes – a review. J. Plant Nutr. Soil Sci. 169:310-329.
Tindall HD (1983). Vegetables in the Tropics. ELBS/Macmillan Education Ltd., Houndmills, Basintoke, Hampshire, UK. pp. 325-337.
Tsai PJ, McIntosh J, Pearce P, Camden B, Jordan BR (2002). Anthocyanin and antioxidant capacity in roselle (Hibiscus sabdariffa L.) extract. Food Res. Int. 35:351-356.
Watanabe FS, Olsen SR (1965). Test of ascorbic acid method for determining phosphorus in water and sodium bicarbonate extract from soils. Soil Sci. Soc. Am. J. 29:677-678.
Wedepohl KH (1995). The composition of the continental crust. Geochim. Cosmochim. Acta 59:1217-1232.
World Meteorological Organization (WMO) (2015) World weather information service. Available at: http://worldweather.wmo.int/en/home.html.