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Sowing date as a determining factor for Roselle, *Hibiscus sabdariffa*, production: II. Effect on biochemical components and quality

Mostafa M. A. El-Sagher[1], Gehan G. Mostafa[2], El-Mowafy A. El-Ghadban[3], Wagdi Saber Soliman[1,*], Abd-Allah Gahory[1]

[1]Department of Horticulture, Faculty of Agriculture and Natural Resources, Aswan University, Egypt. [2]Department of Horticulture (Ornamental Plants), Faculty of Agriculture, Beni Suef University, Egypt. [3]Horticulture Research Institute, Agricultural Research Centre, Cairo, Egypt

Abstract

Roselle plays a pivotal role in the pharmaceutical and food sectors due to its economic and cultural significance. In response to the challenges posed by climate change, identifying the optimal sowing date for Roselle crop in light with climate change has become crucial in agricultural practices. This study aimed to explore the influence of sowing dates on the nutrients, biochemical content, and quality of Roselle in the newly reclaimed soil of Aswan governorate. Four sowing dates were implemented in this experiment including April 15th, April 30th, May 15th and May 30th. The diverse sowing dates resulted in significant differences in photosynthetic pigments and biochemical components such as total phenols, total flavonoids, antioxidant activity, and vitamin C, along with variations in nutrient content (N, P, K, Ca, Fe, and Zn). Notably, sowing on May 15th consistently yielded the highest values for these traits across both seasons. The results underscore that planting on May 15th enhanced the nutritional and biochemical profile of Roselle, contributing to elevated levels of economic and health-related attributes. Specifically, this sowing date significantly improved the biochemical contents and overall quality of Roselle. Consequently, based on the findings of this study, it is recommended to sow Roselle in the middle of May to augment the pharmaceutical properties and quality.

Keywords: Roselle; mineral nutrients; biochemical components; anthocyanin; photosynthetic pigments.

Introduction

Roselle (*Hibiscus sabdariffa* L.) plant is a member of the Malvaceae family, known by various names such as Roselle, hibiscus, and red sorrel in English, and karkadeh in Arabic **[1, 2]**. It is an herbaceous plant, resembling a shrub, typically reaches a height of 1.5-2 meters and is believed to be native to tropical central and West Africa, thriving in tropical and sub-tropical regions **[3]**.

Corresponding author E-mail: wagdi79@agr.aswu.edu.eg
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Roselle cultivation in developing countries is favored due to its ease of growth, adaptability to multi-cropping systems, and versatility for use as both food and fiber. In China, the plant's seeds are valued for their oil content, while the plant itself is utilized for medicinal purposes. In West Africa, the leaves and powdered seeds are incorporated into meals, showcasing the plant's diverse applications. Additionally, Roselle plays a significant role in the pharmaceutical and food industries, underlining its economic and cultural importance **[4]**.

The medicinal value of the Roselle plant has been historically acknowledged **[5]**. Traditionally it is used to address various ailments such as abscesses, bilious conditions, cancer, cough, and fever. Leaves are recognized for their emollient and sedative properties, and the succulent calyx, when boiled in water, is considered a folk remedy for cancer **[6]**. The flowers of Roselle contain gossyperin, anthocyanin, and glycoside hibiscin, suggesting potential diuretic and choleretic effects, blood viscosity reduction, blood pressure lowering, and stimulation of intestinal peristalsis **[7]**. The plant's phenolic compounds contribute to its antimicrobial activities, and it is a rich source of protein, fibers, calcium, iron, carotenes, and vitamin C **[8]**.

In regions with hot summers and mild winters, such as Aswan Governorate in Egypt, Roselle cultivation holds significance. Aswan, with its land reclamation projects aimed at expanding the agricultural area, has a comparative advantage in Roselle production. However, climate change poses a threat to agricultural production in such regions, with challenges like droughts, heat waves, storms, and emerging pests impacting farmers' livelihoods. Future climate predictions indicate a substantial increase in temperature and erratic, intense rainfall patterns, emphasizing the need for climate-resilient and smart agricultural practices for sustainable productivity **[9]**. In response to these challenges, determining the optimal sowing date for Roselle crop has become a vital agricultural practice, especially in recent years.

Against this backdrop, this experiment was designed to explore the nutrient, biochemical components, and quality of Roselle in the newly reclaimed soil of Aswan governorate, focusing on the influence of sowing date.

Materials and methods

The current study took place over the two seasons of 2021 and 2022 at the experimental farm of the Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt (Latitude 24° 05' 53" N., Longitude 32° 53' 57.91" E.). The primary objective was to examine the impact of various sowing dates on the biochemical components and quality of Roselle plants cultivated in newly reclaimed soil.

Plant materials and growth conditions:

Roselle seeds (*Hibiscus sabdariffa* L.) used in this study were procured from the Faculty of Agriculture and Natural Resources, Aswan University. For each of the different sowing dates in both seasons, the seeds were manually sown in the prepared soil, where each sub-plot measured 3×1.5 m and consisted of two rows with a spacing of 30 cm in a row. Approximately 30 days after sowing, the plants were selectively thinned to maintain one plant per hill, resulting in 20 plants per sub-plot (10 plants per row multiply in two rows). To facilitate irrigation, a drip irrigation system was employed, and all other agricultural practices were carried out in accordance with recommended procedures throughout both seasons. The soil texture was identified as sandy, and its physical and chemical characteristics were analyzed using the methods outlined by **[10]** and **[11]**, as detailed in Table (1).

Table 1. The physical and chemical properties of the soil before planting in the two seasons of 2021 and 2022

Soil property	**Season**	
	2021	**2022**
Physical properties		
Clay (%)	3.00	3.50
Silt (%)	0.00	0.00
Sandy (%)	97.00	96.50
Textural class	Sandy	Sandy
Chemical properties		
Soluble cations (mmol/l)		
Ca^{++}	3.06	3.10
Mg^{++}	1.02	1.05
K^{+}	0.83	0.85
Na^{+}	0.76	0.80
Soluble anions (mmol/l)		
CO_3^{--}	0.00	0.00
HCO_3^{-}	7.10	7.06
Cl^{-}	3.60	3.57
SO_4^{--}	0.40	0.44
pH (1:1 soil suspension)	7.64	7.70
EC (dS/cm) at 25°C	0.33	0.32
Available N (mg/kg soil)	128.31	130.00
Available P (mg/kg soil)	8.00	10.00
Available K (mg/kg soil)	175.00	180.00

Experimental design:

The experimental design employed for this study was a randomized complete block in a factorial design, incorporating three replicates. Four sowing dates were considered: April 15th, April 30th, May 15th and May 30th. For the analysis of various characteristics, samples of Roselle plants (comprising 10 plants) were randomly chosen from each sub-plot. Harvesting was carried out on November 1st for both seasons, and the following characteristics were studied:

Photosynthetic pigments:

Samples of fresh leaves were randomly collected from middle parts of the plants for each treatment during flowering stage to evaluate chlorophyll “a”, “b”, and carotenoids **[12].** Leaf pigments were extracted by 80% aqueous acetone and measured using SPECTRO star Nano (BMG LAbTECH GmbH, Germany) at wave length of 663 µm for chlorophyll "a", 644 µm for chlorophyll "b" and 452.5 µm for carotenoids applying the following equations:

Chlorophyll "a" = 10.3 E 663-0.918 E 644 = µg /ml

Chlorophyll "b" = 19.7 E 644-3.87 E 663 = µg /ml

Carotenoids= 4.2 E 452.5 – (0.0264 Chlorophyll "a + 0.426 Chlorophyll "b") = µg/ml

Values of chlorophyll a, b and carotenoids pigments were converted from µg/ml into mg/g fresh weight using the following equation:

mg/g fresh weight = µg/ml × final volume (ml)/ fresh weight of sample (mg)

Determination of total phenolics:

The phenolic content in dried calyxes was determined by employing the Folin-Ciocalteu method, according to the protocol described by **[13]** with modifications. In this procedure, 0.5 g of powdered dried calyxes were resuspended in 20 mL of distilled water. Next, 1 mL of powdered dried calyxes' solution was mixed with 10 mL of distilled water and 500 µL of Folin-Ciocalteu reagent and was allowed to react for 3 mins. Then, 1.5 mL of sodium carbonate (20%, w/v) were added and allowed to stand for an hour. The reading was performed in a UV-VIS Spectrophotometer (Shimadzu-1800) at 765 nm, using distilled water as blank. The results were expressed in mg gallic acid equivalent per gram dry weight sample (mg GAE/g DW).

Determination of total flavonoids:

Total flavonoid contents were determined in dry calyxes by using aluminum chloride colorimetric assay **[14, 15].** About 0.5 mL of the sample solution in methyl alcohol (5 mg/100ml) was mixed with 2 ml of distilled water, and 0.3 ml of $NaNO_2$ (5%). Five min after, 0.3 ml $AlCl_3$ and 2 ml of NaOH (1 M) was added and left for 15 min at room temperature. The solutions were mixed well, and the absorbance was measured at 510 nm against the prepared reagent blank using UV-VIS Spectrophotometer (Shimadzu-1800). Total flavonoid content was expressed as mg catechin equivalents (CE)/100 g dry weight.

Antioxidant activity:

Antioxidant activity was measured according to **[16]** by means of the diphenyl-picrylhydrazyl (DPPH) radical degradation method.

Vitamin C:

Vitamin C of dried calyxes was measured spectrophotometrically (Hitachi, U-1800, Tokyo, Japan) by measuring Fe^{2+} complexes with 2, 2-dipyridyl **[16]** and reading the absorbance of the sample solution at 525 nm.

Total anthocyanin content:

At harvesting time, total anthocyanin content was determined in air-dried Roselle calyxes according to the pH differential method described by **[17].** Samples of calyxes were randomly collected and air dried under shade conditions. Known weight (200 mg) of calyxes were extracted using extraction solution (15 ml methanol 75% acidic with hydrochloric acid 1%), grinded in Chinese mortar, incubated in the refrigerator at 4°C in the dark overnight, filtered, then reextracted with 10 ml extraction solution. The buffer solution (sodium acetate, pH 4.5) was added to 1 ml extraction, and the buffer solution (potassium chloride, pH 1.0) was added to another 1 ml extraction. The absorption of anthocyanin was calorimetrically measured at both pH at 520 and 700 nm using Spectrophotometer (SPECTROstar Nano, BMG LAbTECH GmbH, Germany). The absorbance was measured within 20–50 min of preparation, and acidic methanol was used as blank.

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

$$\text{Total anthocyanin (mg/g DW)} = A \times MW \times WF \times 1000/ \varepsilon \times 1$$

Where A = (A510 nm −A700 nm) pH 1.0 − (A510 nm− A700 nm) pH 4.5; MW (molecular weight) = 449.2 g/mol; DF =dilution factor; 1 = cuvette path length in cm; ε = 26900 L/mol.cm which is the molar extinction coefficient for cyanidin 3-O-β-D-glucoside. 1000= factor to convert g to mg. The measurements were performed using a UV-Visible spectrophotometer (Optizen Pop, Mecasys -Korea).

Mineral contents:

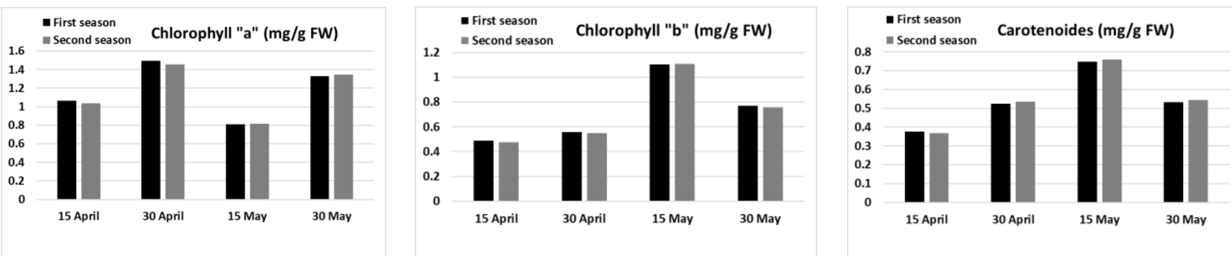
For chemical analysis, dried plant leaves (0.2 g) were wet-digested with concentrated H_2SO_4:H_2O_2 (1:1, v/v) using a heating digester (DK, Velp Scientific Srl, Italy). The extracts were used for chemical analysis. Nitrogen (%) was determined using the Microkjedhal method according to the methodology of **[18].** Phosphorus (%) was determined by the soft digestion method using ammonium molybdate and ascorbic acid by colorimetric method using a UV-VIS Spectrophotometer (Shimadzu-1800) **[19]**. Potassium (%) was estimated using a flame-photometer according to **[20]**. Calcium (%) was determined using an atomic absorption spectrophotometer. The flame photometer was applied for calcium (Ca) determination according to the method described by **[21].** Iron (Fe) and zinc (Zn) were determined on aliquots of the solutions of the ash were established according to the method of **[22]** using Atomic Absorption Spectrophotometer, Perkin-Elmer Model 2380 manufacture (USA).

Statistical analysis:

The data obtained from the experiment underwent statistical analysis utilizing the "F" Test as described by **[23]**. Post hoc comparisons of means were conducted using the least significant difference (L.S.D.) test following the methodology outlined by **[24]**. The entire statistical analysis was executed using the Statistix 8.1 program.

Results

Statistically, it was found that sowing date has a significant effect on photosynthetic pigments content during the two seasons (Figure 1). Planting on the April 30th date showed maximum chlorophyll "a" (1.493 and 1.459 mg/g FW), while the lowest one (0.811 and 0.817 mg/g) was associated with those planted on May 15th in the 1st and 2nd seasons, respectively. In contrast, the highest values of chlorophyll "b" content (1.102 and 1.108 mg/g FW) were registered with planting on May 15th, while the least ones (0.489 and 0.477 mg/g FW) were noticed with planting on April 15th in the 1st and 2nd seasons, respectively. In the same line with chlorophyll "b", the higher carotenoids content (0.747 and 0.758 mg/g FW) was recorded with the 3rd planting date (May 15th), while the least (0.377 and 0.367 mg/g FW) was in the 1st planting date (April 15th) in the 1st and 2nd seasons, respectively.



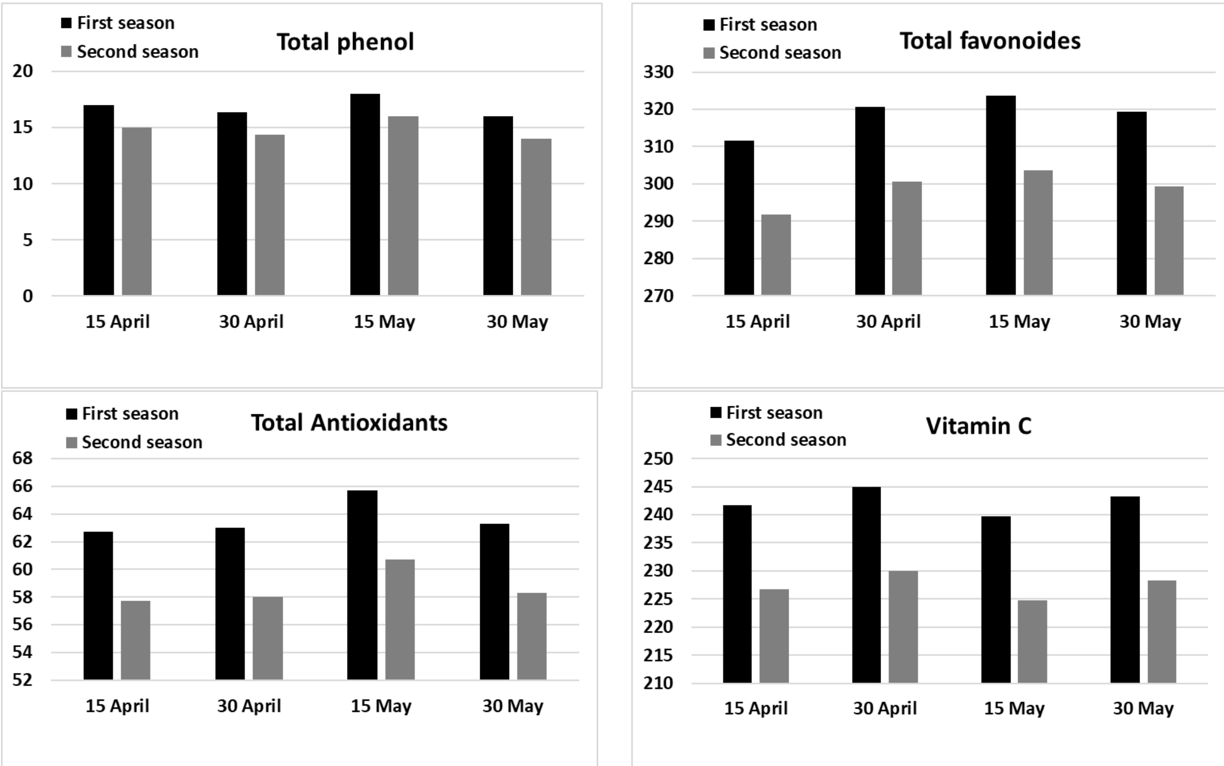
Figure(1): Effect of sowing dates on chlorophyll "a", "b" and carotenoids content of Roselle leaves.

The influences of sowing date on total phenolic content of Roselle calyxes were significant during 2021 and 2022 seasons as presented in Figure (2). It is obvious from the figure that sowing on May 15th produced the highest values of total phenolic content (18 and 16 TPC mg/g), while sowing on May 30th resulted in the lowest ones (16 and 14 TPC mg/g) in the 1st and 2nd seasons, respectively.

Statistically, it was found that sowing date has a significant effect on total flavonoid during the two seasons (Figure 2). Sowing on May 15th date showed maximum total flavonoid (323.7 and 303.7 mg/100g), while the lowest one (311.7 and 291.7 mg/100g) were associated with those planted on April 15th in the 1st and 2nd seasons, respectively.

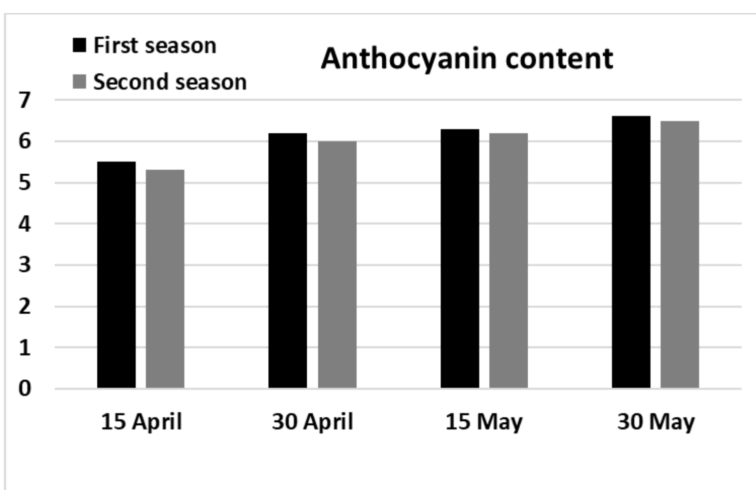
Also, analysis of variance proved significant differences due to the effects of sowing date on antioxidant activity of Roselle during the two seasons. Similar to total flavonoids, the highest percentage of antioxidant activity (65.7 and 60.7 %) was recorded with the 3rd planting date (May 15th), while the least one (62.7 and 57.7 %) was in the 1st planting date (April 15th) in the 1st and 2nd seasons, respectively.

The influences of sowing dates on vitamin C content of Roselle sepals were significant during the two seasons of 2021 and 2022 seasons as presented in Figure (2). In the same line of total flavonoids and antioxidants activity, sowing on May 15th produced the highest values of vitamin C content (239.7 and 224.7 mg/100g FW), while sowing on April 15th resulted in the lowest ones (241.7 and 226.7 mg/100 g FW) in the 1st and 2nd seasons, respectively.



Figure(2): Effect of sowing dates on phytochemical contents; total phenols, total flavonoides, total antioxdants and vitamine C content of Roselle calyxes.

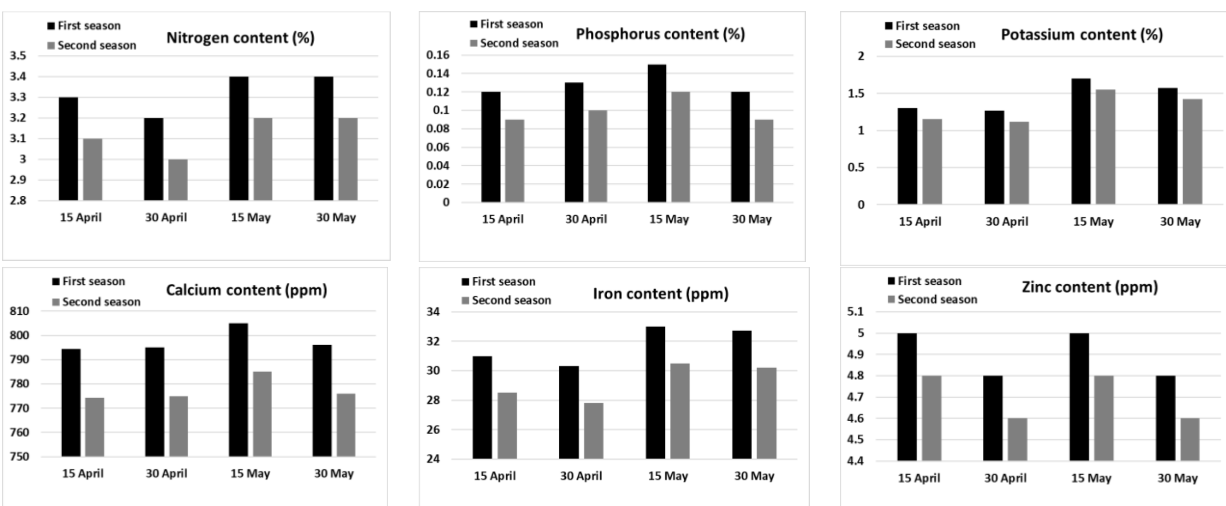
Figure (3) represented the mean value of total anthocyanins content of Roselle as affected by sowing date. Analysis of variance revealed significant differences among different planting dates in the total anthocyanins content during the two studied seasons. The highest values of total anthocyanins content (6.6 and 6.5 mg/g dry weight) were registered with sowing on May 30th, while the least ones (5.5 and 5.3 mg/g dry weight) were noticed with planting on April 15th in the 1st and 2nd seasons, respectively.



Figure(3): Effect of sowing dates on anthocyanin content of Roselle calyxes.

Data in Figure (4) presented the effect of sowing dates on mineral nutrients contents in Roselle leaves during the two seasons of 2021 and 2022. Obviously, all sowing dates were significantly affected nutrients percentages in leaves during both seasons. The highest N percentages (3.4 and 3.2%) were obtained from planting on May 15th or May 30th with significant increment compared to the other dates. Meanwhile, the highest P percentages (0.15 and 0.12 %) were obtained from planting on May 15th with significant increment compared to the other dates, in the 1st and 2nd seasons, respectively. In the same line, the highest K percentages (1.7 and 1.55 %) were obtained from planting on May 15th with significant increase compared to the other dates in the 1st and 2nd seasons, respectively.

Data in Figure (4) also showed the effect of sowing dates on Ca content in Roselle' leaf during 2021 and 2022 seasons. Visibly, all planting dates significantly affected Ca contents in Roselle leaves during both seasons. Higher Ca content (805 and 785 mg/100g) was obtained from planting on May 15th with significant increase compared to the other dates in the 1st and 2nd seasons, respectively. Visibly, all sowing dates were also significantly affected Fe contents in Roselle leaves during both seasons. Higher Fe content (33 and 30.5 mg/100g) was obtained from planting on May 15th with significant increase compared to the other dates in the 1st and 2nd seasons, respectively. In term of Zn content, the highest value of the Zn content (5 and 4.8 mg/100g) was due to planting on April 15th and May 15th in the 1st and 2nd seasons, respectively.



Figure(4): Effect of sowing dates on mineral nutrients content (N, P, K, Ca and Zn) of Roselle leaves.

Discussion

Considering the economic significance of the Roselle plant, particularly in Aswan Governorate, which stands as the primary producer of this medicinal plant in Egypt, it raises a critical need to enhance not only its growth and productivity but also its biochemical content and quality. To address these concerns, the current study was carried out at the experimental farm of the Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt. The primary focus was to investigate the performance of the Roselle plant when grown under different sowing dates in newly reclaimed sandy soil. In this discussion, we delve into the impact of the studied factor on the biochemical and nutrient content, as well as the overall quality of Roselle plant.

The influences of sowing date on different biochemical substances content i.e. photosynthetic pigments, total phenolic compounds, total flavonoid, antioxidant activity, vitamin C, total anthocyanins, and leaf nutrients concentrations of Roselle plants were significant during both seasons. It is obvious from the results that planting on May 15th produced the highest values of these traits during both seasons. Similar result was noticed by **[25-33].** Roselle is sensitive to daylength, requiring a short day of 12 to 13 hours **[34].** The appropriate planting date has an effect on the various chemical components of Roselle plant, and we are in the same line with of previous researches. It was shown through these studies that planting dates have an impact on vegetative growth, fruit productivity, N, P, K, acidity, anthocyanin and fixed oil in seeds **[30, 31, 32, 33, 35, 36, 37, 38; 39]**.

Conclusion

In this study, we systematically assessed the impact of diverse sowing dates on the nutrient, biochemical, and overall quality of roselle plants. Our findings reveal significant effects associated with different sowing dates, particularly in relation to photosynthetic pigments, biochemical components (such as total phenols, total flavonoids, antioxidants activity, and vitamin C), mineral nutrient levels, and the quality as reflected by anthocyanin content, of the Roselle plant. Notably, the results underscored that sowing plants on the 15th of May hold a distinctive advantage, significantly enhancing both the economic and health-related values of Roselle. This enhancement was attributed to improvements in biochemical contents and overall quality. As a result, based on the insights gained from this study, we advocate for the strategic practice of sowing Roselle plants in the middle of May as a recommended approach to amplify both the pharmaceutical properties and overall quality of this valuable plant.

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