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SEAWEED EXTRACT AND BALANCED FERTILIZER EFFECT ON THE MEDICINAL COMPOUNDS OF ROSELLE (*HIBISCUS SABDARIFFA* L.)

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SUMMARY

The following study aimed to investigate the effects of applying different concentrations of seaweed extract and balanced fertilizer on the medicinal active compounds in roselle (*Hibiscus sabdariffa* L.), as carried out during the growing season of 2023 at the Kerbala Governorate, Kerbala, Iraq. Employing a randomized complete block design with three blocks and two factors, the first factor was foliar spraying with balanced fertilizer at concentrations of 0, 2.5, and 5 ml L^{-1}. Meanwhile, the second factor was foliar application with seaweed extract at concentrations of 0, 0.25, 0.5, and 0.75 ml L^{-1}. Results showed significant differences among the spraying treatments with balanced fertilizer. The 5 ml L^{-1} spray treatment excelled in the concentration of quercetin, gossypetin, hibiscetin, protocatechuic acid, and sabdaretin in cup leaves, with averages of 0.282, 0.247, 0.190, 0.267, and 0.128 mg g^{-1}, respectively. The seaweed extract concentrations also had a significant effect on the content of active compounds. Its enhanced concentration (0.75 ml L^{-1}) showed increased values of active compounds, i.e., 0.349, 0.291, 0.235, 0.312, and 0.163 mg g^{-1}, respectively. Notably also, the interaction between the factors has a remarkable effect on all the traits under study.

Keywords: Roselle (*H. sabdariffa* L.), balanced fertilizer, seaweed extract, biologically active compounds, growth and yield traits

Key findings: The balanced fertilizer foliar application (5 ml L^{-1}) enhanced the ratios of medicinal active components as compared with other fertilizer treatments in roselle (*H. sabdariffa* L.). The active compounds also significantly gained improvement with the foliar spraying of seaweed extract at an increased concentration (0.75 ml L^{-1}). The balanced fertilizer (5 ml L^{-1}) and seaweed extract (0.75 ml L^{-1}) produced the highest averages for all the medicinal components.

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INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is a plant of substantial medicinal importance, primarily due to the therapeutic properties of its sepals, which are a rich source of the glycoside, hibiscin, and various bioactive compounds, including protocatechuic acid (PCA). PCA is recognizably a potent antioxidant with the potential to treat certain types of cancer (Kilic *et al.,* 2011). The current global trend emphasizes the adoption of environmentally friendly agricultural practices to ensure sustainable food production. However, the high cost, inconsistent availability, and frequent adulteration of inorganic fertilizers explored the present challenges that can disrupt the soil ecosystem balance (Babalola *et al.,* 2016). Additionally, traditional fertilizer application methods, commonly used by farmers worldwide, including in Iraq, often lead to significant nutrient losses through leaching and volatilization, thereby reducing the crop plants' nutrient uptake.

Foliar nutrient application has been increasingly operational as an alternative to conventional soil fertilization to enhance the nutrient use efficiency and mitigate environmental impacts (Keshinro *et al.,* 2023). Among the essential macronutrients, nitrogen (N), phosphorus (P), and potassium (K) are crucial for plant growth and development. Nitrogen, being a macronutrient, is vital for stem and leaf growth and is one of the most demanded nutrients by crops. Similarly, phosphorus plays a key role in root development, flower and fruit formation, and accelerating the crop maturity, making it essential for overall plant growth. Potassium enhances plant strength and resilience against adverse environmental conditions and is integral to numerous physiological processes (Fahmy and Hassan, 2019). Abbas and Ali's (2011) findings demonstrated that foliar application of NPK (1 and 2 g L^{-1}) significantly improved specific growth parameters in roselle cultivars. The NPK (2 g L^{-1}) resulted in the highest average anthocyanin content and vitamin C content compared with the control treatment.

The seaweed extract is an emerging valuable alternative for foliar feeding, gaining popularity due to its potential to reduce dependence on chemical fertilizers. These extracts have demonstrated significant effects on improving both the quantity and quality of crop yields (Khan *et al.,* 2009). Seaweed extracts are rich in nutrients and essential growth stimulants, such as auxins, cytokinins, and betaines, as well as organic acids, amino acids, and vitamins that influence various plant biological activities (Sharma *et al.,* 2014). Furthermore, seaweed extracts enhance plant growth by stimulating root development, increasing chlorophyll content, promoting vegetative branching, and inducing earlier flowering, ultimately leading to higher crop yields (Elansary *et al.,* 2016). A study by Al-Tai (2017) on roselle revealed the seaweed extract with 15 ml L^{-1} notably outperformed other treatments, with results in the highest concentrations of the medicinally active compounds quercetin and hibiscetin compared with the control treatment.

Given the medicinal and economic significance of roselle and the limited field and pharmaceutical research on this plant, the presented study sought to explore the safe and natural alternative to chemical fertilizers for enhancing roselle growth and yield. Specifically, the said research investigated the effects of different concentrations of balanced fertilizer and seaweed extract on the active compounds produced by roselle through physiological processes.

MATERIALS AND METHODS

A field experiment on roselle (*H. sabdariffa* L.) commenced during the summer of 2023 under field conditions at the Ibn-al-Baitar Vocational Preparatory School, Iraq. The study objective was to investigate the response of roselle plants to varying concentrations of balanced fertilizer and seaweed extract. The experiment layout was in a randomized complete block design (RCBD) involving two factors and three replications. The first factor consisted of foliar application of balanced fertilizer at concentrations of 0, 2.5, and 5 ml L^{-1}. The second factor involved foliar spraying with seaweed extract at concentrations of 0, 0.25, 0.5, and 0.75 ml L^{-1}.

The content measurement of biologically active compounds in roselle sepals proceeded. Initially, the preparation of a roselle sepal extract followed the method described by Kelly *et al.* (1995). One gram of dried, powdered sepals received 100 ml of acetonitrile. Then, the mixture's placement in an ultrasonic device remained for 30 min at a temperature of 35 °C. The solution's subsequent filtering used a 0.45-micron microfilter before transferring to sealed glass containers for further analysis*.* Subsequently, the injection of 25 microliters of the prepared solution succeeded into a high-performance liquid chromatography (HPLC) system to identify and quantify the active compounds, viz., quercetin, gossypetin, hibiscetin, protocatechuic acid, and sabdaretin in the roselle sepals. The compounds' separation had their concentrations determined by comparison with reference standards using the following equation (Table 1)***:***

Statistical analysis

The collected data underwent statistical analysis based on the experimental design. The treatment means' comparison used the least significant difference ($LSD_{0.05}$) test. The conducted analyses employed the SAS software, following the methodology outlined by Al-Rawi and Khalafallah (2000).

RESULTS AND DISCUSSION

Quercetin

The results indicate a significant increase in the quercetin content inside the roselle sepals as a result of balanced fertilization spraying (5 ml L^{-1}) and achieved the highest average (0.282 mg g^{-1}), compared with distilled water spraying (0.254 mg g^{-1}) (Table 2). This notable increase was likely due to the enhanced effectiveness of the balanced fertilizer components. Nitrogen, in particular, promotes the leaf area expansion, leading to greater light interception by the leaves, which eventually improves photosynthetic efficiency. Consequently, this improved photosynthesis supports the production of various secondary metabolites and compounds within the sepals, including quercetin, a key bioactive component in roselle (Fageria *et al.,* 2009).

Table 1. Retention time and solution area of active substances in the sepals of the roselle plant.

No.	Standard	Retention time (min)	Area
1	Quercetin	1.83	17687
2	Hibiscetin	5.87	21648
3	Gossypetin	3.18	29458
4	Protocatechuic acid	6.92	9027
5	Sabdaretin	3.96	11347

Table 2. Effect of seaweed extract, balanced fertilizer, and their interactions on the quercetin content in roselle sepals.

Seaweed	Balanced Fertilizer (ml L^{-1})			Means (mg g^{-1})
(ml L^{-1})	0	2.5	5	
0	0.196	0.204	0.220	0.206
0.25	0.215	0.251	0.246	0.237
0.50	0.282	0.290	0.291	0.287
0.75	0.325	0.352	0.372	0.349
Means (mg g^{-1})	0.254	0.274	0.282	

$LSD_{0.05}$ Seaweed = 0.003466, Balanced Fertilizer = 0.003001, Interactions = 0.006003

Moreover, the findings showed a significant difference among the seaweed extract concentrations for quercetin content, and the seaweed extract (0.75 ml L^{-1}) recorded the highest average (0.349 mg g^{-1}) versus the lowest average obtained in the control treatment (0.206 mg g^{-1}) (Table 2). The pronounced effect of seaweed extract on quercetin content can refer to its positive role in enhancing the leaf area, thereby improving photosynthetic efficiency and subsequently boosting the production of secondary metabolites in the sepals (Harhash *et al.*, 2024).

The interaction between seaweed extract and balanced fertilizer concentrations also revealed meaningful differences for quercetin content. However, the highest quercetin content (0.372 mg g^{-1}) appeared with the interaction of balanced fertilizer (5 ml L^{-1}) and seaweed extract (0.75 ml L^{-1}), compared with the control treatment for both factors, which showed the lowest average (0.196 mg g^{-1}).

Gossypetin

The results indicated a significant effect of the balanced fertilizer on the concentration of gossypetin content. The balanced fertilizer spraying with 5 ml L^{-1} achieved the highest average (0.247 mg g^{-1}), compared with the distilled water application, which recorded the lowest average (0.192 mg g^{-1}) (Table 3). This positive impact of balanced fertilizer could be because of the essential nutrients it provides, which are crucial for plant growth. These elements stimulate various physiological processes, including nutrient uptake and the synthesis of carbohydrates and proteins, leading to increased leaf area and enhanced enzyme activity. As a result, the production of compounds, such as amino acids, sugars, and lipids, rose, ultimately promoting nutrient transport and increasing the concentration of biologically active substances within roselle sepals (El-Dissoky *et al.,* 2020).

The seaweed extract concentrations also revealed significant differences for the gossypetin content, and the seaweed concentration (0.75 ml L^{-1}) showed the premier average (0.291 mg g^{-1}), compared with the control treatment (0.159 mg g^{-1}) (Table 3). The observed increase in gossypetin concentration with seaweed spraying (0.75 ml L^{-1}) can be attributable to enhanced shoot growth, which positively affects the various physiological processes during plant development. The seaweed extract supplies the plant with essential nutrients for tissue development and improves the photosynthetic efficiency, leading to a higher concentration of active substances. Additionally, adequate light exposure during the growth period may contribute to sepal coloration and compound accumulation (Tsai *et al.,* 2002).

Similarly, the interaction between seaweed extract and balanced fertilizer concentrations proved to be significant. The interaction of balanced fertilizer (5 ml L^{-1}) and seaweed extract (0.75 ml L^{-1}) showed the topmost average of gossypetin content (0.332 mg g^{-1}), compared with the control treatment for both factors, which recorded the lowest average (0.141 mg g^{-1}).

Table 3. Effect of seaweed extract, balanced fertilizer, and their interactions on the gossypetin content in roselle sepals.

Seaweed (ml L^{-1})	Balanced Fertilizer (ml L^{-1})			Means (mg g^{-1})
	0	2.5	5	
0	0.141	0.145	0.169	0.159
0.25	0.162	0.174	0.228	0.188
0.50	0.211	0.248	0.259	0.239
0.75	0.257	0.286	0.332	0.291
Means (mg g^{-1})	0.192	0.213	0.247	
$LSD_{0.05}$ Seaweed: 0.01082, Balanced Fertilizer: 0.00937, Interactions: 0.01875				

Table 4. Effect of seaweed extract, balanced fertilizer, and their interactions on the hibiscetin content in roselle sepals.

Seaweed (ml L^{-1})	Balanced Fertilizer (ml L^{-1})			Means (mg g^{-1})
	0	2.5	5	
0	0.093	0.098	0.130	0.107
0.25	0.121	0.138	0.171	0.143
0.50	0.144	0.178	0.218	0.180
0.75	0.231	0.233	0.242	0.235
Means (mg g^{-1})	0.147	0.161	0.190	
$LSD_{0.05}$ Seaweed: 0.00646, Balanced Fertilizer: 0.00559, Interactions: 0.01118				

Hibiscetin

Noteworthy variations were evident for hibiscetin content in roselle sepals due to various concentrations of balanced fertilizer. The balanced fertilizer spraying with 5 ml L^{-1} recorded the highest average content (0.190 mg g^{-1}), superior to distilled water, which recorded the lowest average of hibiscetin content (0.147 mg g^{-1}) (Table 4). The pronounced effect of balanced fertilizer could have resulted from the catalytic action of its elements in stimulating plant growth. These elements enhance the plant cell growth by influencing various physiological processes, leading to cell division and increased efficiency of carbon assimilation, which consequently boosts metabolite production, including hibiscetin (Patil and Chetan, 2018).

The seaweed extract concentrations also significantly affected the hibiscetin content in roselle leaves, and the concentration of 0.75 ml L^{-1} gave the highest average (0.235 mg g^{-1}), compared with the control treatment, recording the lowest average (0.107 mg g^{-1}) (Table 4). The superior effect of the seaweed concentration (0.75 ml L^{-1}) can likely be due to its positive role in enhancing the leaf area. This, in turn, positively influenced the concentration of secondary metabolites produced by the plants by enhancing the efficiency of carbon assimilation and increasing the formation of enzymes responsible for the biosynthesis. Additionally, the seaweed extract provides various elements that are fundamental components of these active compounds (Allen and Pilbeam, 2006). Likewise, the interaction of both factors exhibited a significant effect, and the maximum hibiscetin content (0.242 mg g^{-1}) emerged with the interaction of balanced fertilizers (5 ml L^{-1}) and seaweed extract concentration (0.75 ml L^{-1}), compared with the control treatment for both factors. The latter recorded the minimum average in roselle sepals (0.093 mg g^{-1}).

Protocatechuic acid

The results showed significant differences among the spraying treatments of balanced fertilizer for the protocatechuic acid in roselle sepals (Table 5). The balanced fertilizer spraying with 5 ml L^{-1} achieved the supreme average of protocatechuic acid (0.267 mg g^{-1}), while the lowest average resulted in the distilled water at 0.213 mg g^{-1}. This superiority can be because of the positive role of the balanced fertilizer in providing plants with essential nutrients to enhance their vegetative growth. These nutrients promote meristematic cell division and growth, as well as the expansion of leaf surface area. These factors positively influenced the production of carbohydrates and proteins in the leaves, which are fundamental for building plant tissues and, consequently, raising the concentration of active compounds in roselle sepals (Gomaa *et al.,* 2018).

On the seaweed extract effect, outcomes indicated the concentration of 0.75 ml L^{-1} was superior by giving the highest average of protocatechuic acid (0.312 mg g^{-1}), while the lowest average came from the control treatment (0.172 mg g^{-1}) (Table 5). The superiority of seaweed extract in this context is ascribable to its vital role in enhancing the plant's response to

Table 5. Effect of seaweed extract, balanced fertilizer, and their interactions on the protocatechuic acid in roselle sepals.

Seaweed (ml L^{-1})	Balanced Fertilizer (ml L^{-1})			Means (mg g^{-1})
	0	2.5	5	
0	0.162	0.165	0.188	0.172
0.25	0.183	0.194	0.248	0.208
0.50	0.231	0.268	0.273	0.259
0.75	0.277	0.307	0.353	0.312
Means (mg g^{-1})	0.213	0.233	0.267	
$LSD_{0.05}$ Seaweed: 0.01072, Balanced Fertilizer: 0.00935, Interactions: 0.01972				

Table 6. Effect of seaweed extract, balanced fertilizer, and their interactions on the sabdaretin content in roselle sepals.

Seaweed (ml L^{-1})	Balanced Fertilizer (ml L^{-1})			Means (mg g^{-1})
	0	2.5	5	
0	0.068	0.080	0.092	0.080
0.25	0.082	0.096	0.109	0.096
0.50	0.119	0.129	0.133	0.127
0.75	0.152	0.157	0.179	0.163
Means (mg g^{-1})	0.105	0.116	0.128	
$LSD_{0.05}$ Seaweed: 0.003466, Balanced Fertilizer: 0.003002, Interactions: 0.006003				

environmental stresses, stimulating the production of particular acids as part of its defense mechanisms, including protocatechuic acid (Hanafy *et al.,* 2022). Furthermore, the interaction of balanced fertilizer (5 ml L^{-1}) and seaweed extract (0.75 ml L^{-1}) occurred with the highest average of protocatechuic acid (0.353 mg g^{-1}) versus the control treatments of both factors, recording the lowest average in roselle sepals (0.162 mg g^{-1}).

Sabdaretin

Study findings demonstrated considerable differences among the balanced fertilizer doses for sabdaretin content inside roselle sepals (Table 6). The balanced fertilizer spraying (5 ml L^{-1}) showed the highest average of sabdaretin content (0.128 mg g^{-1}), while spraying with distilled water gave the lowest average (0.105 mg g^{-1}). The positive influence of the higher concentration of balanced nutrients on plants may be because of increased internal plant activity, enhanced photosynthesis, and elevated production of secondary metabolites (Taiz and Zeiger, 2006).

On the effect of seaweed extract spraying, the concentration of 0.75 ml L^{-1} appeared to be superior, with an average sabdaretin content of 0.163 mg g^{-1}. Meanwhile, the distilled water recorded the lowest average (0.080 mg g^{-1}) (Table 6). The better effect of seaweed extract on sabdaretin content within the sepals could be due to its constituents' role in enhancing the enzymes' activity involved in secondary metabolic pathways within the plant, leading to an increased production. Additionally, the seaweed extract stimulates the expression of genes associated with secondary metabolism, thereby promoting the synthesis and storage of secondary compounds within roselle sepals, including sabdaretin (Belhaj *et al.,* 2020).

The interaction between both factors, seaweed extract and balanced fertilizer concentrations, revealed significant differences for sabdaretin content. The interaction of balanced fertilizer (5 ml L^{-1}) and seaweed concentration (0.75 ml L^{-1}) displayed the maximum average (0.179 mg g^{-1}) versus the control treatments of both factors, which showed the minimum average (0.068 mg g^{-1}).

CONCLUSIONS

The study results suggested a synergistic effect of the seaweed extract and balanced fertilizer, where their combined application resulted in superior outcomes than their individual use. The results demonstrated varying concentrations of seaweed extract and balanced fertilizer had a remarkable impact on the biologically active compounds of the roselle plant, highlighting the potential benefits of integrating these treatments for optimal plant growth and metabolite production.

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