



Effect of cytokinin on growth parameters, yield, and medicinally active compounds of roselle (*Hibiscus sabdariffa* L.) under water stress conditions

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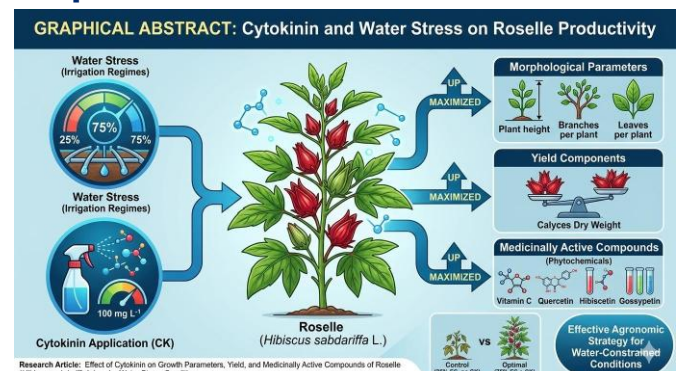
Abstract

Introduction: Water stress significantly limits the agricultural and medicinal potential of roselle (*Hibiscus sabdariffa* L.). Exogenous plant growth regulators, such as cytokinins, may mitigate these adverse environmental effects and enhance overall plant productivity. Objective: To investigate the impact of cytokinin application and varying water stress levels on the vegetative growth, yield, and medicinally active compounds of roselle. Methods: A factorial field experiment using a Randomized Complete Block Design (RCBD) with three replications was conducted during the spring season of 2025 in Al-Mussaib, Iraq. Treatments consisted of three cytokinin levels (0, 50, and 100 mg L⁻¹) and four water stress levels (25%, 50%, 75%, and 100% of field capacity). Data were statistically analyzed using the Least Significant Difference (LSD) test. Results: The application of 100 mg L⁻¹ cytokinin significantly maximized plant height (95.08 cm), branches (9.20 plant⁻¹), leaves (106.70 plant⁻¹), capsules (38.83 plant⁻¹), and calyces' dry weight (9.30 g). It also peaked the concentrations of vitamin C, quercetin, hibiscetin, and gossypetin. Similarly, irrigation at 75% field capacity produced the highest mean values for the same traits. Crucially, the interaction between 100 mg L⁻¹ cytokinin and 75% field capacity recorded the absolute highest values across all studied morphological, yield, and phytochemical parameters. Conclusions: Applying 100 mg L⁻¹ of cytokinin optimally enhances the growth, yield, and phytochemical composition of *Hibiscus sabdariffa* L., particularly when combined with

moderate water management (75% field capacity). This combination provides an effective agronomic strategy for cultivating this medicinal crop under constrained water conditions.

Keywords: Hibiscus; Cytokinins; Water Stress; Crop Yield; Ascorbic Acid; Quercetin.

Graphical Abstract



Source: Own authorship.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is a significant species of the Malvaceae family and has many common names across the globe, such as Roselle, Jamaica and Karkadeh. It is also known as Karkadeh in Egypt [1]. It is a plant of tropical Africa and is widely spread in many tropical areas of the world [2]. It is cultivated mostly in the southern provinces of Iraq, including Al-Qadisiyah Governorate [3]. Roselle is an annual or

biennial plant grown for its stems, fibre, leaves, flowers, seeds and particularly its red fleshy calyces which enclose the fruits [4].

Roselle is an economic crop due to the presence of extracts that are used in preparing refreshing drinks from its red calyces [5]. Calyces are also an important source of glycoside hibiscin which has several medicinal properties; such as reducing the blood viscosity, strengthening cardiac muscles, calming nervous system and lowering high blood pressure [6]. Plant growth regulators are plant hormones that play important roles in the regulation of physiological processes, such as gibberellins and cytokinins. They are important in seed germination, protecting leaf senescence by inhibiting protein and chlorophyll degradation, boosting nucleic acid biosynthesis and increasing protein biosynthesis [7].

Among the physiological parameters measured, black cumin plant height, number of branches, number of leaves, leaf area, fresh and dry plant weight, and leaf carbohydrate content were significantly increased due to treatment with gibberellic acid (20 ppm) according to [7]. Likewise, three growth regulators, i.e., coconut milk, gibberellin and ethephon were evaluated by [8] and it was found that the use of gibberellin significantly enhanced the number of branches, stem weight and total dry weight of the black cumin plant and root.

Plant growth and development is not random, but is tightly controlled by plant hormones which allow plants to sense and respond to environmental conditions so keeping the growth factors in the environment in balance with genetically controlled growth processes. Because of the relatively low productivity of roselle in Iraq, studies focused on improving vegetative growth characteristics are of particular importance, since vegetative growth characteristics are source-related traits that can favorably affect the sink characteristics, and therefore enhance yield performance [9].

In this context, there is a strong linkage between increasing crop productivity and crop tolerance to environmental constraints with sustainable agricultural practices. In recent years, the beneficial effect of biological and organic fertilization along with micronutrients such as boron on the chemical properties of soil and yield characteristics of crops has been emphasized [10-13]. Such sustainable soil management practices are especially critical in reducing abiotic stresses and enhancing degraded or desertified soils' agricultural capacity [14].

The effects of growth regulators on yield components and active compounds of black cumin was

investigated by [15]. They found that the balanced application of the treatments significantly increased the number of capsules per plant, capsule weight, number of seeds per capsule, weight of 1000 seeds, number of seeds per capsule, and accumulation of biologically active compounds.

Reduced irrigation rates (50% and 25% of field capacity) have been previously demonstrated to significantly affect most growth and yield parameters. The reduction could be due to insufficient water levels at critical growth phases, resulting in physiological and enzymatic disturbances which have adverse effects on plant performance. In these conditions, the plants gradually lose their ability to absorb water, and then enter into the temporary wilting and permanent wilting stages [16-18].

The better performance under the 75% and 100% of field capacity irrigations indicates that the plants could take up a larger proportion of water or they could prevent a drop in the water potential of their tissues in the initial stages of drought. This adaptive response is commonly linked to the formation of compatible solutes like soluble sugars by the process of osmotic adjustment, which helps plants to sustain cellular processes during water stress and helps them to rapidly recover after stress relieving [16,18].

In addition, polygenetic control of plant tolerance to water stress is common, affecting a combination of adaptive mechanisms, such as morphological, physiological, and biochemical changes. Water stress may impair photosynthetic activity both directly by decreasing the efficiency of the carbon assimilation in the leaves and indirectly by causing stomatal closure. Therefore, leaf area is reduced as a response to the adaptive measures to reduce transpiration water loss, especially when plant growth regulators are used to reduce stress effects [16,17]. According to [19] foliar application of putrescine resulted in higher plant resistance to soil moisture deficiency and was found to have a significant effect on the growth and yield parameters studied.

The present study was thus carried out to assess growth, yield and quality response of roselle to water stress and to explore the possibility of using cytokinin application to overcome the water stress conditions.

Materials and Methods

A field experiment was conducted in Al-Mussaib Great Project area, Al-Wutayfiyah Village in Iraq, between 44°22' E longitude and 32°36' N latitude, to test the effect of cytokinin and water stress on some vegetative growth, yield and quality attributes of

roselle (*Hibiscus sabdariffa* L.). The experiment had been planned as a Randomized Complete Block Design (RCBD) with three replications with the following factors. The first factor was 3 concentrations of the plant growth regulator Cytokinin namely 0, 50 and 100 mg L⁻¹. The second factor consisted of four irrigation regimes, based on different soil moisture levels of 25%, 50%, 75% and 100% field capacity. The number of replications (three) per treatment and sample size (18) were statistically calculated to have adequate degrees of freedom to estimate experimental variance and detect significant differences among treatments with the LSD test.

The N fertilizer used was urea (46% N) applied in two equal amounts at 100 kg ha⁻¹. The first application was 2 weeks after planting and second was 1 month after planting. Triple super phosphate (16% P₂O₅) was applied as phosphorus fertilizer at a rate of 80kg/ha and included during land preparation. For optimal plant growth, phosphorus management is important because the dynamics of phosphorus adsorption and release are greatly influenced by soil chemical properties and by organic management [20]. The physical and chemical properties of the soil were determined following the procedures explained by Page et al. [21] and local roselle cultivar seeds were planted. The chemical and physical properties of experimental soil are given in Table 1.

Seeding was done on March 15, 2025. The seeds were planted in hills 25 cm apart with 3 to 4 seeds planted in each hill and the ridges were 75 cm apart. After emergence, seedlings were thinned to one plant per hill when plants reached a height of 10–15 cm, resulting in a final plant population density of 53,333 plants ha⁻¹. All recommended agronomic practices, such as irrigation, hoeing and pest control practices were carried out as required throughout the growing season.

On harvest, 10 plants were randomly sampled in each experimental unit and the following plant characters were recorded: plant height (cm), number of branches per plant, number of leaves per plant and number of capsules per plant. The calyces were separated from the capsules and air dried to measure calyx dry weight. The dried calyces were then analysed for vitamin C content and the concentration of the bioactive compound's quercetin, hibiscetin and gossypetin using the methods described by [6,22].

The collected data was analysed statistically, and the means of the treatments were compared using Least Significant Difference (LSD) test at 5% probability level as detailed by Al-Rawi and Khalaf Allah [23].

Table 1. Physical and Chemical Properties of the Experimental Soil.

Property	Unit	Value
Sand	g kg ⁻¹ soil	481.4
Silt	g kg ⁻¹ soil	325.2
Clay	g kg ⁻¹ soil	193.4
Soil texture	—	Loam
Electrical conductivity (EC)	dS m ⁻¹	2.08
Soil pH	—	7.6
Available nitrogen (N)	mg kg ⁻¹	32.4
Available phosphorus (P)	mg kg ⁻¹	9.82
Available potassium (K)	mg kg ⁻¹	137.1
Organic matter	g kg ⁻¹	8.1
Cation exchange capacity (CEC)	cmol kg ⁻¹ soil	18.7

Source: Own authorship.

Results and Discussion

Plant Height (cm)

From the results shown in Table 2 it is clear that the plant height is affected by the studied factors significantly. The application of cytokinin at rate of 100 mg L⁻¹ significantly increased the plant height and the largest mean value of 95.08 cm was obtained as compared to that of untreated control with the lowest mean value of 82.38 cm. As far as water stress treatments is concerned, the mean plant height of 75% F.C. was 94.73 cm and was significantly higher than the mean plant height of 25% F.C. which was 78.83 cm. There were no significant differences between the 75% and 100% field capacity treatments. Interactions between cytokinin and water stress also were significant. Plants grown with 100mg of cytokinin with 75% field capacity were the tallest with an average height of 102.5 cm. The untreated plants under 25% field capacity had the lowest plant height with an average of 72.8 cm.

Cytokinin acts as a growth promoter for the height of the plants; this might be due to its ability to stimulate cell division and increase meristematic activity, thereby inducing stem elongation and vegetative growth. Cytokinin also postpones the senescence of leaves and promotes mobilization of nutrients and photosynthetic efficiency, which leads to greater production of assimilates and plant growth. The higher performance seen for the 75% field capacity treatment could be related to soil moisture availability to sustain physiological processes without inducing excessive soil water conditions. With the high-water stress conditions (25% field capacity), a decrease in water availability is likely to have limited

cell growth, nutrient uptake and photosynthetic activity, leading to shorter plants.

Table 2. Effect of Cytokinin and Water Stress on Plant Height (cm) of Roselle (*Hibiscus sabdariffa* L.)

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	72.8	78.5	85.2	78.83
50	81.4	86.9	91.3	86.53
75	88.2	93.5	102.5	94.73
100	87.1	91.8	101.3	93.40
Mean	82.38	87.68	95.08	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	3.25	3.89	7.03	

Note: Prepared by the authors based on experimental findings.

Number of Branches per Plant

From the results in Table 3, it is observed that there were significant differences among the treatments in effecting the number of branches per plant. Application of cytokinin at 100 mg L⁻¹ showed significant increment in branching and produced the highest value of 9.20 branches plant⁻¹ as compared to control treatment with the lowest value of 7.48 branches plant⁻¹.

With regard to water stress treatments, irrigation at 75% field capacity produced the highest mean of 9.43 branches plant⁻¹ and irrigation at 25% field capacity produced the lowest mean of the 6.90 branches plant⁻¹. There were no significant differences found between the 75% and 100% field capacity treatments. The effect of interaction of cytokinin and water stress levels was significant. The cytokinin 100 mg L⁻¹ in combination with 75% Field Capacity gave the most branches per plant, 10.7 branches plant⁻¹. The treatment with 25% FC, however, had the highest value of 6.4 branches plant⁻¹.

The cytokinin-induced promotion of branching number may be related to its known ability to break apical dominance and promote lateral bud growth. Cytokinin stimulates meristematic tissue to divide, and stimulates the growth of axillary buds causing branching. Furthermore, adequate soil water at 75% FC may have enhanced the uptake of nutrients and photosynthetic activity, which resulted in having adequate assimilates for branch growth. On the contrary, the severe water stress caused less vegetative growth due to limited cell expansion and metabolic activity thus lessening the number of branches produced per plant.

Table 3. Effect of Cytokinin and Water Stress on the Number of Branches per Plant of Roselle (*Hibiscus sabdariffa* L.).

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	6.4	6.9	7.4	6.90
50	7.2	7.8	8.5	7.83
75	8.2	9.4	10.7	9.43
100	8.1	9.2	10.2	9.17
Mean	7.48	8.33	9.20	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	0.56	0.73	1.14	

Note: Prepared by the authors based on experimental findings.

Number of Leaves per Plant

The results of the cytokinin application and water stress levels shown in Table 4 indicated significant differences among the treatments with respect to the number of leaves per plant. The cytokinin treatment at 100 mg L⁻¹ gave a significantly higher leaf production and the highest mean value of 106.70 leaves plant⁻¹ than the control treatment with the lowest mean value of 93.13 leaves plant⁻¹.

Regarding the irrigation treatments, the plants irrigated at 75% of field capacity, yielded the highest mean (106.77 leaves plant⁻¹) while the plants irrigated at 25% of field capacity yielded the lowest mean (89.07 leaves plant⁻¹). There were no significant differences between the 75% field capacity and 100% field capacity treatments. Additionally, there was an interaction effect between cytokinin and water stress levels. The application of cytokinin (100 mg L⁻¹) with 75% field capacity resulted in the highest number of leaves (115.6 leaves per plant). The lowest value (85.2 leaves plant⁻¹) was observed for the control treatment under 25% field capacity.

The stimulatory activity of cytokinin on leaf formation might be explained by its ability to promote cell division, to stimulate the growth of shoots and to delay senescence of leaves by maintaining chlorophyll and protein levels. Cytokinin also stimulates the movement of nutrients towards growing tissues, leading to more leaf initiation and growth. This could be because sufficient moisture is available for maintaining the metabolic processes, photosynthesis and nutrient uptake under 75% field capacity. Under Water Deficit Conditions, on the other hand, turgor loss of cells decreases the leaf initiation and expansion, and speeds up senescence, leading to a smaller number of leaves/plant.

Table 4. Effect of Cytokinin and Water Stress on the Number of Leaves per Plant of Roselle (*Hibiscus sabdariffa* L.).

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	85.2	88.4	93.6	89.07
50	91.3	96.7	103.4	97.13
75	98.6	106.1	115.6	106.77
100	97.4	104.7	114.2	105.43
Mean	93.13	98.98	106.70	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	3.83	4.22	7.36	

Note: Prepared by the authors based on experimental findings.

Number of Capsules per Plant

The data presented in Table 5 revealed that water stress level and/or cytokinin application had significant effects on the number of capsules per plant. Application of cytokinin at 100 mg L⁻¹ showed significant increase in capsule production with a highest mean value of 38.83 capsules plant⁻¹ when compared with control treatment that presented lowest mean value of 30.60 capsules plant⁻¹.

As far as irrigation treatments are concerned, the 75% field capacity treatment had the greatest mean capsule number of 38.60 capsules plant⁻¹ while the 25% field capacity treatment had the lowest mean of 28.50 capsules plant⁻¹. There were no significant differences between the 75 and 100 field capacity treatment. Water stress x cytokinin interaction had significance. The treatment with 100 mg L⁻¹ cytokinin and 75% Field capacity gave the maximum number of capsules per plant (43.5 capsules plant⁻¹). The lowest value obtained was for the plants grown under 25% field capacity, where the untreated plants had the lowest value of 26.2 capsules plant⁻¹.

The stimulatory action of cytokinin on capsule formation could be due to its stimulatory effect on vegetative development, number of branches and leaves, and its stimulatory effect on photosynthetic efficiency, which would lead to more assimilates being available for the reproductive development. Cytokinin also has been reported to stimulate flower initiation and inhibit flower and fruit abortion, thereby increasing the number of flower capsules. Moderate soil moisture conditions appeared to favor flowering, pollination and fruit set, as reflected in the superior performance at 75% field capacity. In contrast, severe water stress probably caused limitations in photosynthesis, nutrient uptake, and assimilate translocation which led to fewer capsules per plant.

Table 5. Effect of Cytokinin and Water Stress on the Number of Capsules per Plant of Roselle (*Hibiscus sabdariffa* L.).

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	26.2	27.5	31.8	28.50
50	28.4	33.9	37.2	33.17
75	34.1	38.2	43.5	38.60
100	33.7	37.6	42.8	38.03
Mean	30.60	34.30	38.83	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	1.42	1.65	2.92	

Note: Prepared by the authors based on experimental findings.

Dry Weight of Roselle Calyces (g Plant⁻¹)

Table 6 illustrated that significant difference was found among the different treatments studied with respect to dry weight of roselle calyces. The application of cytokinin at 100 mg L⁻¹ strongly boosted calyx dry weight with the highest mean value of 9.30 g whereas the lowest mean value of 7.38 g was observed in case of control treatment. In terms of irrigation treatments, the highest mean dry weight of calyces was observed at 75% F.C (9.47 g) while the lowest was observed at 25% F.C (6.90 g). There was no difference between the 75% and 100% field capacity treatments.

The factor of cytokinin by water stress was significant. The dry weight of calyx from the treatment with 100 mg L⁻¹ cytokinin at 75% field capacity was the highest at 10.8 g, whereas the treatment with 25% field capacity (control) had the lowest value of 6.3 g. Cytokinin effect on calyx dry weight might be explained by its stimulating effect on vegetative growth, photosynthetically activity and assimilation accumulation. Cytokinin stimulates cell division, postpones senescence and increases the translocation of photosynthates from source organs to developing reproductive organs, resulting in increased biomass production in the calyx's. The higher performance recorded for plants watered at 75% FC might be related to the fact that they received adequate soil moisture level that allows the plants to keep their physiological functions and production of assimilates without causing high water stress. However, moisture deficiency at 25% FC may have slowed dry matter production and nutrient uptake and caused a decrease in calyx dry matter.

Table 6. Effect of Cytokinin and Water Stress on Dry Weight of Roselle Calyces (g Plant⁻¹)

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	6.3	6.8	7.6	6.90
50	6.9	7.8	8.5	7.73
75	8.2	9.4	10.8	9.47
100	8.1	8.9	10.3	9.10
Mean	7.38	8.23	9.30	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	0.47	0.58	0.92	

Note: Prepared by the authors based on experimental findings.

Vitamin C Content in Roselle Calyces (mg g⁻¹)

As can be seen in Table 7, the results showed that the different treatments studied had significantly different effects on the vitamin C content in roselle calyces. The vitamin C concentration was significantly high following the application of Cytokinin at 100 mg L⁻¹ with vitamin C mean value of 34.33 mg g⁻¹ compared to the minimum mean value of 25.00 mg g⁻¹ obtained under control treatment. As for irrigation regimes, the highest mean value of Vitamin C content (35.13 mg g⁻¹) was obtained from the 75% field capacity treatment whereas the lowest mean value (27.28 mg g⁻¹) was obtained from the 25% field capacity treatment. There were no differences between the 75% and 100% field capacity treatments.

There was a strong interaction between cytokinin and water stress. Vitamin C content that was highest for 100 mg L⁻¹ cytokinin + 75% field capacity was 40.8 mg g⁻¹, while the treatment with 25% field capacity was the lowest (23.5 mg g⁻¹). The cytokinin-induced rise in vitamin C levels could be attributed to its effects on boosting metabolic activity, increasing photosynthetic efficiency, and decreasing the loss of ascorbic acid due to oxidative degradation by regulating antioxidant defense systems. Cytokinin can also reduce the senescence of the tissues, thus keeping bioactive compound levels in the tissues high. The superior performance under 75% field capacity suggests that moderate water availability supports optimal enzymatic activity and biosynthetic pathways involved in ascorbic acid accumulation. In contrast, severe water stress likely accelerates oxidative stress and metabolic disruption, leading to reduced vitamin C content in calyces.

Table 7. Effect of Cytokinin and Water Stress on Vitamin C Content in Roselle Calyces (mg g⁻¹)

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	23.5	24.9	26.6	25.00
50	25.8	28.3	31.5	28.53
75	30.2	33.7	41.6	35.17
100	29.6	32.6	40.8	34.33
Mean	27.28	29.88	35.13	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	2.68	3.14	5.36	

Note: Prepared by the authors based on experimental findings.

Quercetin Content in Roselle Calyces (mg g⁻¹)

The findings in Table 8 indicated that there were significant differences between the studied treatments in terms of results of quercetin content present in the calyces of roselle. The highest mean value of quercetin (0.380 mg g⁻¹) was obtained with Cytokinin application at 100 mg L⁻¹, while the lowest mean value of quercetin (0.329 mg g⁻¹) was obtained from control treatment. With regard to the treatments applied in irrigation, the mean quercetin content of 0.390 mg g⁻¹ was obtained in treatment 75% field capacity, while the mean of 0.296 mg g⁻¹ was obtained in treatment 25% field capacity. There were no differences between the 75% and 100% field capacity treatments.

The effect of cytokinin and water stress was significant. The highest concentration of quercetin (0.421 mg g⁻¹) was recorded when 100 mg L⁻¹ cytokinin was applied at 75% field capacity, while the lowest concentration (0.286 mg g⁻¹) was obtained for the control with 25% field capacity. Cytokinin-induced higher levels of quercetin could be the result of the ability of cytokinin to increase the metabolic activity and de-repress the enzymes of the phenylpropanoid biosynthetic pathway. Cytokinin also can decrease senescence and oxidative degradation which may help preserve the higher concentration of flavonoids. Better performance at moderate water availability (75% FC) indicates that moderate water availability is optimal for enzymatic activity and carbon partitioning for secondary metabolism. At the other end of the spectrum, with severe water stress, the metabolic efficiency is lowered and the availability of precursors is restricted, leading to less accumulation of quercetin.

Table 8. Effect of Cytokinin and Water Stress on Quercetin Content in Roselle Calyces (mg g⁻¹)

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	0.286	0.294	0.308	0.296
50	0.311	0.332	0.358	0.334
75	0.365	0.408	0.433	0.402
100	0.352	0.398	0.421	0.390
Mean	0.329	0.358	0.380	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	0.021	0.032	0.051	

Note: Prepared by the authors based on experimental findings.

Hibiscetin Content in Roselle Calyces (mg g⁻¹)

Table 9 revealed significant differences between the effects of the studied treatments on the amount of hibiscetin found in roselle calyces. The application of cytokinin was found to be significantly higher compared to the control treatment in terms of hibiscetin concentration, with the highest mean value of 0.408 mg g⁻¹ and the lowest mean value of 0.336 mg g⁻¹, respectively. When the irrigation treatments were compared, the 75% field capacity had the highest mean amount of hibiscetin content (0.408 mg g⁻¹), and the 25% field capacity treatment had the lowest mean value (0.293 mg g⁻¹). There were no significant differences between the 75% and 100% field capacity treatments.

The cytokinin/water stress interaction was significant. The 100 mg L⁻¹ cytokinin and 75% field capacity treatment gave the highest hibiscetin content of 0.454 mg g⁻¹ while the treatment under 25% field capacity gave the lowest of 0.265 mg g⁻¹. This cytokinin-induced rise in the level of hibiscetin could be explained by the stimulation of secondary metabolic pathways and better physiological activity, resulting in more flavonoids being synthesized. Cytokinin also plays a role in preserving cell integrity and postponing senescence, which are associated with increased production of bioactive compounds. The positive response at 75% field capacity indicates a balance between the availability of water and enzyme activity during the synthesis of phenolics, whereas the plant under severe water stress conditions experiences a decline in metabolic activity and/or availability of precursors.

Table 9. Effect of Cytokinin and Water Stress on Hibiscetin Content in Roselle Calyces (mg g⁻¹)

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	0.265	0.293	0.321	0.293
50	0.334	0.351	0.385	0.357
75	0.374	0.411	0.473	0.419
100	0.371	0.398	0.454	0.408
Mean	0.336	0.363	0.408	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
	0.041	0.052	0.087	

Note: Prepared by the authors based on experimental findings.

Gossypetin Content in Roselle Calyces (mg g⁻¹)

The results obtained from the studied treatments as shown in Table 10 showed that significant differences existed in the effect of these treatments on the gossypetin content of roselle calyces. The highest mean value of gossypetin (0.199 mg g⁻¹) was observed in the 100 mg L⁻¹ of Cytokinin application and the lowest mean value of gossypetin (0.110 mg g⁻¹) was observed in the control treatment.

As far as irrigation treatments are concerned, the highest mean value of gossypetin content (0.191 mg g⁻¹) was obtained with treatment at 75% field capacity while the lowest mean value (0.089 mg g⁻¹) was recorded at 25% field capacity. The difference between the 75% field capacity and 100% field capacity treatments were not significant. Cytokinin × Water stress interaction was significant. The highest gossypetin content (0.248 mg g⁻¹) was obtained in the treatment with 100 mg L⁻¹ cytokinin at 75% field capacity while the lowest was 0.074 mg g⁻¹ in the treatment of 25% field capacity.

Enhanced biosynthesis of secondary metabolites might be related to the increased amount of gossypetin under cytokinin application, as well as to better physiological performance under optimal growth conditions. Cytokinin could also play a possible role in the maintenance of metabolic balance and the synthesis of flavonoid derivatives. The positive growth effect at 75% FC indicates that moderate water stress would be conducive to phenols production and enzymatic activity, while severe water stress would decrease the biosynthetic efficiency and the accumulation of secondary metabolites.

Table 10. Effect of Cytokinin and Water Stress on Gossypetin Content in Roselle Calyces (mg g⁻¹)

Water Stress (%)	Growth Regulator (mg L ⁻¹)			Mean
	0	50	100	
25	0.074	0.087	0.106	0.089
50	0.107	0.142	0.185	0.145
75	0.132	0.182	0.258	0.191
100	0.128	0.167	0.248	0.181
Mean	0.110	0.145	0.199	
LSD (P ≤ 0.05)	Growth Regulator	Water Stress	Interaction	
		0.027	0.038	0.062

Note: Prepared by the authors based on experimental findings.

Discussion

When cytokinin was applied to roselle (*Hibiscus sabdariffa* L.), significant differences were noted between it and the control in all vegetative growth, yield and quality parameters, especially at the highest concentration of 100mgL⁻¹. This marked improvement is directly linked with the basic physiological functions of plant growth regulators such as cytokinins and gibberellins, which serve as signaling molecules controlling the differentiation and division of cells. Cytokinins induce axillary bud outgrowth, mobilize nutrients to the active sink, and inhibit the degradation of essential proteins and chlorophyll pigments and delay leaf senescence [7]. Cytokinins preserve the integrity of the chlorophyll molecules and extend the functional photosynthetic window of leaves, which boosts the accumulation and production of carbohydrates.

In addition to this, the exogenous cytokinin treatment triggers the production of nucleic acids and boosts the protein biosynthesis rate, leading to strong vegetative growth such as increased plant height, number of branches and leaf area [24].

The results are quite consistent with the results obtained by Mousa et al. [7] and Atiyah et al. [8] and Al-Mamouri [15] who reported that the optimal levels of growth regulators resulted in considerable improvement in the morphological and yield parameters of the medicinal plants related to black cumin.

There were also significant differences between irrigation regimes for all of the investigated characteristics. The irrigation treatment that was maintained at 75% of field capacity (FC) produced the largest mean values for all growth and yield parameters compared with the adjacent irrigation treatment, 100% FC. On the other hand, water deficits at 25% and 50% FC resulted in significant reduction in plant performance. It is mainly due to limited water

availability in the rhizosphere, thus the efficiency of water absorption by the roots, which causes water deficits in plants internally. In such moisture stress, critical physiological and enzymatic processes are severely impaired, resulting in reduction of turgor pressure in cell, inhibition of cell elongation and inducing temporary or permanent wilt stages [18].

The superior efficiency of roselle in 75% and 100% FC shows that the plant is able to adapt to maintain a favourable and stable internal water potential in relation to the external water potential. This stability is obtained either by extracting moisture from the deeper layers of the soil efficiently or by controlling the moisture consumption in the early stages of a dry spell. When moisture is adequate or the stress is moderate, plants are able to activate the osmotic adjustment system to build up compatible osmolytes, such as soluble sugars and free proline. These solutes help to maintain the functional stomatal conductance and also protect cellular structures, as well as enable rapid physiological recovery upon end of stress [16,18].

Water stress response in plants is a multifaceted process involving multi-genic networks, which coordinate the process of morphological, physiological and biochemical adaptations [16,17]. Water stress has a negative impact on photosynthetic capacity in two ways: (1) indirectly by causing stomatal closure to limit transpirational water loss and (2) directly by decreasing biochemical efficiency of carbon assimilation in the mesophyll cells. This means that the total leaf area is reduced evolutionary as a way to reduce total evapotranspiration surface [17].

Yet, water stress could also be used to trigger the transition of the primary metabolic pathways to secondary metabolic pathways. This change promotes bioactive secondary metabolites biosynthesis and accumulation, such as quercetin, hibiscetin and gossypetin, which act as protective antioxidants, scavenging the reactive oxygen species (ROS) produced under stress [26].

To reduce these negative impacts, the use of exogenous growth regulators and subsequent use of a specialized nutrient solution via the foliage has become a very effective management technique. Exogenous cytokinin helps the plant adjust to soil moisture limitations by re-establishing hormonal balance, improving plant root to shoot communication, maintaining stomatal function and maintaining chloroplast ultrastructure under stress [19].

Moreover, the introduction of such innovative foliar applications such as bio-stimulants, micronutrients and liquid organic formulations greatly

boosts the plant's own defense system, nutrient uptake efficiency and yields quality even under varying environmental stress [27-31].

Finally, the result of this research demonstrates that roselle is highly sensitive to soil moisture stress and that strategic foliar application of cytokinin is a promising farming practice to reduce soil moisture stress damages and thus ensure the plant's growth, maximize the calyx production and increase the concentration of valuable medicinal compounds.

Study Limitations

This research showed that cytokinin has a positive effect under water stress conditions, but the results are only applicable to one local roselle cultivar, grown under agro-climatic conditions, in one season. Replication with additional locations is recommended to validate these findings.

Conclusion

The study showed that the cytokinin application to leaves at 100 mg L⁻¹ was statistically significant to produce improved growth, yield and medicinally active compounds in roselle (*Hibiscus sabdariffa* L.). It resulted in improvement of plant height, number of branches, number of leaves, number of capsules and calyx dry weight, besides improving the contents of vitamin C, quercetin, hibiscetin and gossypetin. All of the traits studied were also significantly affected by water stress. The result showed that there was an optimum soil moisture condition (75% field capacity) for both vegetative growth and yield, while bioactive compound accumulation was achieved. The moderate soil moisture level (75% field capacity) resulted in the best performance compared to 25% and 50% field capacity treatments, showing that moderate soil moisture is suitable for vegetative growth, yield formation and accumulation of bioactive compounds. When the interaction of cytokinin at 100 mg L⁻¹ and 75% field capacity was considered, the highest values for most of the studied traits were obtained. It means that application of cytokinin with moderate irrigation could alleviate the adverse impacts of water stress and enhance the productivity and medicinal value of roselle plants.

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Author contributions: Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Project administration; Supervision; Writing - original draft, and Writing-review & editing- All authors.

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Ethical approval

Not applicable. This study does not involve human or animal subjects. All experimental research and field studies on plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

Informed Consent

Not applicable.

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Data Sharing Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The author declares that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Similarity Check

It was applied by Ithenticate®.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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