

Response of mineral content characteristics of Halwani Grapevine leaves to spraying with the growth regulators Kinetin, Brassinolide and Seaweed Extract

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Abstract :

This study was conducted in a vineyard belonging to the Agricultural Research and Experiment Station at the College of Agriculture, Kirkuk University, Iraq, during the 2025 growing season. The aim was to determine the effect of spraying with the growth regulators Kinetin at three concentrations (0, 75, and 150) mg L⁻¹, Brassinolide at three concentrations (0, 0.75, and 1.5) mg L⁻¹, and Seaweed extract at two concentrations (0 and 3) ml L⁻¹ on the mineral content of two-year-old Halwani grapevines. The spraying was carried out in three applications during the growing season, from May 1st to June 11th, with a 21-day interval between each application. The results obtained can be summarized as follows: Foliar spraying with kinetin at concentrations of 75 and 150 mg L⁻¹ resulted in a significant increase in the percentage of nitrogen and protein in the leaves, reaching 4.834%, 4.773%, 30.210%, and 29.828%, respectively. Spraying with 150 mg L⁻¹ of kinetin resulted in a significant increase in the percentage of phosphorus, potassium, carbohydrates, and the C/N ratio in the leaves, reaching 0.466%, 1.037%, 12.569%, and 2.682%, respectively. Spraying with brassinolide at concentrations of 0.75 and 1.5 mg L⁻¹ significantly increased the leaf nitrogen and protein content to 4.784%, 4.834%, 29.919%, and 30.210%, respectively. Spraying at a concentration of 0.75 mg L⁻¹ also significantly increased the leaf phosphorus, potassium, and total carbohydrate content to 0.447%, 1.041%, and 11.982%, respectively. Spraying with a seaweed extract at a concentration of 3 ml L⁻¹ significantly increased the leaf nitrogen and protein content to 4.927% and 30.791%, respectively. The two-way and three-way interaction of the study agents significantly improved the studied characteristics. Conclusion: Foliar application of Kinetin, Brassinolide, and seaweed extract, individually or combined, significantly enhanced the mineral profile and vegetative traits of 'Halwani' grapevines. This improvement effectively accelerated vine development, notably shortening the period required to reach the fruiting stage.

Keywords: Kinetin, Brassinolide, Seaweed Extract, Halwani Grapevine, *Vitis vinifera* L.

Introduction:

Grape *Vitis vinifera* L. is one of the oldest and most economically important fruit crops worldwide, due to its content of many elements, sugars, amino acids, nutrients, and compounds represented by anthocyanins and phenolic proteins [1]. It is believed that

its cultivation began in Central Asia in the region between the southern Black Sea and the Caspian Sea, and it is widely distributed in subtropical and temperate regions between latitudes (20-50) north of the equator and (20-40) south of the equator. Iraq is considered one of the oldest places famous for its cultivation, as history

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indicates that it dates back to the Assyrian era before 2440 BC [2]. Grape leaves are vital parts of the plant, representing the primary site of photosynthesis. Here, carbon dioxide is fixed and carbohydrates necessary for plant growth and development are formed. They also serve as a major source of nutrients transported to the plant's consuming organs, such as the fruit and roots, directly impacting growth efficiency and productivity. Furthermore, the leaves play a crucial role in regulating transpiration and water balance, in addition to transporting minerals within the plant, which positively affects its physiological state [3]. The Halwani variety is among the best late-maturing table grape varieties available and is highly sought after in Iraq. It is characterized by its large, almost conical, elongated clusters with a single shoulder, and large, attractive, spherical berries that are reddish-purple, sometimes with a hint of purple depending on sun exposure. The berries have a thin skin and a fleshy, juicy pulp [4,2].

Growth regulators are compounds that may be natural, produced by the plant itself, or synthetic, prepared in factories. Synthetic growth regulators play an important role in regulating the vital processes of the plant, as they perform functions similar to those performed by plant hormones. However, they are not considered plant hormones in the strict sense, but are known as growth regulators, these compounds affect biochemical processes within the plant at very low concentrations, which is reflected in its growth, development, and the unfolding of its parts [5]. Kinetin is one of the cytokinins that are produced naturally within the plant, but it can be added externally to plants to play an important and essential role in cell division, the building of nucleic acids (DNA and RNA), differentiation of vascular tissues, flower formation, delaying aging, and increasing chlorophyll content [6]. In this field, [7] showed that spraying six-year-old Halawn

and Black Balad grapevines with the growth regulator KT-30 at a concentration of 0.50 mg L⁻¹ increased the leaf content of the macronutrient NPK (1.32, 0.148 and 1.88) % respectively compared to concentrations of 0 and 0.25 ml L⁻¹. [8] found that spraying grape seedlings of the 'Shidda Bayda' variety with cytokinins at concentrations of (0, 25, 50, and 100) mg L⁻¹ resulted in a significant increase in leaf nitrogen. In a study by [9] on the effect of spraying pomegranate trees of the 'Salimi' cultivar with three concentrations of cytokinins, they found that a concentration of 150 mg L⁻¹ improved tree growth characteristics compared to concentrations of (0 and 75) mg L⁻¹.

Brassinolide is a plant hormone that plays a significant role in plant growth and development. It is known as a crude fatty extract from the pollen of the rapeseed plant (*Brassica napus*), Brassinolide has effects similar to auxins, gibberellins, and cytokinins in influencing physiological processes such as elongation, cell division, vascular differentiation, flowering, senescence, and stress tolerance. It is also involved in the biosynthesis of cell wall components, DNA, RNA, and proteins, and is essential for metabolic activities in plants [10]. [11] demonstrated that spraying ten-year-old pomegranate trees of the Salimi variety with three concentrations (0, 0.15, and 0.3) mg L⁻¹ resulted in a significant improvement in some studied chemical characteristics. The 0.3 mg L⁻¹ concentration yielded the highest percentage of nitrogen and phosphorus content in the leaves. [12] concluded in their study of the effect of foliar spraying with Brassinosteroids at four concentrations (0, 0.5, 1 and 2) mg L⁻¹ on Canino apricot trees that the 2 mg L⁻¹ concentration was significantly superior in the content of nitrogen, phosphorus and potassium in the leaves for both seasons of the study compared to the other concentrations.

Seaweed extract is used, after drying or extraction, as a mineral source for plants due to its high content of growth-promoting substances, in addition to amino acids, nutrients, and vitamins [13]. It also has significant physiological effects on plants when sprayed or added to the soil, increasing the plant's tolerance to salinity and drought. It is considered an organic resource used in agricultural production at a rate of 15 million tons annually worldwide [14]. In their study, [15] found that spraying four-year-old Anna apple trees with Algae Tecamin extract at four concentrations (0, 2, 4, and 6) ml L⁻¹ resulted in a significant increase in the N/C ratio in the leaves compared to the other concentrations. [16] found that spraying 5-year-old Shatter Al-Anz grapevines with a 40 ml L⁻¹ seaweed extract significantly improved their growth characteristics compared to concentrations of (0 and 20) ml L⁻¹. Given the scarcity of studies in this area, particularly on young Halwani grapevines in Kirkuk, this study aimed to increase the mineral content of grapevines by spraying with the growth regulators kinetin and brassinolide, along with the seaweed extract. It also sought to determine the optimal levels of each of the individually studied factors and to identify the best combination of them.

Material and Methods

This study was conducted in the vineyard located at the Agricultural Research and Experiment Station of the College of Agriculture/University of Kirkuk, Iraq, during the 2025 growing season, located at longitude 44.22 East and latitude 35.33 North, at an altitude of 300 m above sea level, from 1/5/2025 to 1/9/2025, on two-year-old grapevines planted at a distance of (2 m between vines and 2.5 m between rows) and trained by wire training. The entire field was cleaned of weeds, grasses, and plant debris. Summer pruning of the vines was carried out twice, the first at the beginning of July 2025 and the second at

the beginning of August, with the aim of strengthening the stem and roots and increasing and stimulating growth. The foliage of the vines was sprayed with three concentrations of Kinetin (0, 75 and 150) mg L⁻¹, three concentrations of Brassinolide (0, 0.75 and 1.5) mg L⁻¹, and two concentrations of Seaweed Extract (0 and 3) ml L⁻¹ containing 0.10% total nitrogen and 0.44% soluble potassium (K₂O). A 20-liter knapsack sprayer was used to thoroughly wet the vines, and the spraying was carried out at sunset. The vines were sprayed on three separate dates: the first on May 1st, the second on May 21st, and the third on June 11th, with a 20-day interval between sprays. This was done for all factors during the season with a one-day interval between sprayings. A 0.1% surfactant was added to the spray solution to reduce the surface tension of the leaves. The experiment was designed as a completely randomized block design with three replicates and three factors, with two vines per experimental unit. This resulted in 36 vines per replicate and a total of 108 vines. Treatments were randomly distributed within each block. The data were statistically analyzed using an SAS program ANOVA table and computer-aided analysis of variance [17]. Means were compared using Duncan's Multiple Range test at a probability level of 0.05, as described by [18].

Studied characteristics: Fully grown leaves with an average of 8 leaves were collected from different areas and from all trees in the experimental units on August 28, 2026. They were washed several times with distilled water to remove any adhering dust and placed in perforated paper bags inside an electric oven at a temperature of (70°C ± 5°C) for 48 hours or until a constant weight was reached. The leaves were then thoroughly ground, and 0.2 g of the dry sample was taken and digested using concentrated sulfuric acid (H₂SO₄) and perchloric acid (HClO₄) at a ratio of 1:4 ml each, respectively, according to the method

described by [19]. The following characteristics were studied:

- 1- Nitrogen (%): Nitrogen was estimated using a Micro-Kjeldahl apparatus according to the method described by [20].
- 2- Phosphorus (%): Phosphorus was estimated using the colorimetric method and light absorption reading at a wavelength of 410 nm using an EMC V LAB-1 100 spectrophotometer, according to the method of [21].
- 3- Potassium (%): Potassium was estimated using an Elicocl-378 flame photometer, according to the method of [21].
- 4- Carbohydrates (%): Total carbohydrates were estimated using an EMC V LAB-1 100 spectrophotometer at a wavelength of 490 nm, according to the method of [22].
- 5- C/N ratio in leaves: This was calculated by dividing the percentage of total carbohydrates by the percentage of nitrogen in the leaves.
- 6- Protein percentage in leaves (%): Calculated according to the following equation: Protein percentage = Nitrogen percentage $\times$ 6.25.

Results and Discussion:

Nitrogen content in leaves (%): The results obtained in Table (1) indicate that the percentage of nitrogen in leaves was significantly affected by the levels of spraying with the growth regulator kinetin. The highest percentage of nitrogen in leaves was obtained when spraying at a concentration of 75 mg L⁻¹, reaching 4.834%, compared to the control treatment.

Spraying with the growth regulator brassinolide also had a significant effect; spraying at a concentration of 1.5 mg L⁻¹ resulted in a significant increase in this characteristic, reaching 4.834%, compared to the control treatment. Spraying with seaweed extract resulted in the highest percentage at a concentration of 3 ml L⁻¹, reaching 4.927%, compared to the lowest concentration. Regarding the two-way interaction between the growth regulators kinetin and brassinolide, the interaction treatment of 75 mg L⁻¹ kinetin combined with 0.75 mg L⁻¹ brassinolide resulted in a 5.356% increase in this trait compared to the other interactions, the dual interaction treatment of 75 mg L⁻¹ kinetin combined with 3 ml L⁻¹ seaweed extract showed a significant increase in the same trait, reaching 5.301% compared to the other interactions. The interaction between the growth regulator brassinolide at concentrations of 0.75 and 1.5 mg L⁻¹ combined with the 3 ml L⁻¹ seaweed extract spray treatment also resulted in a significant increase in this trait, reaching 4.984% and 4.945%, respectively, compared to the distilled water spray treatment. The three-way interaction between the growth regulator kinetin, brassinolide, and seaweed extract resulted in the highest effect: the treatment with a concentration of (150 mg L⁻¹ of kinetin + 1.5 mg L⁻¹ of brassinolide + 0 ml L⁻¹ of seaweed extract), reaching 5.34%. This did not differ significantly from the treatment with a concentration of (75 mg L⁻¹ of kinetin + 1.5 mg L⁻¹ of brassinolide + 3 ml L⁻¹ of seaweed extract), which reached 5.363%, compared to the other treatments.

Table 1. Effect of spraying with Kinetin, Brassinolide, and Seaweed Extract and their interactions on the nitrogen content (%) in the leaves of young Grapevines cv. Halwani

Kinetin (mg L ⁻¹)	Brassinolide (mg L ⁻¹)	Seaweed Extract (ml L ⁻¹)		Kinetin $\times$ Brassinolide
		0	3	
0	0	3.498 e	4.662 b-d	4.080 e

	0.75	4.165 d	5.390 a	4.778 dc
	1.5	4.403 d	4.403 d	4.403 de
75	0	3.199 e	5.308 a	4.254 e
	0.75	5.478 a	5.233 ab	5.356 a
	1.5	4.420 d	5.363 a	4.891 bc
150	0	5.186 ab	4.581 cd	4.883 bc
	0.75	4.128 d	4.328 d	4.228 e
	1.5	5.343 a	5.070 a-c	5.207 ab
	Kinetin			Average Kinetin
Kinetin × Seaweed Extract	0	4.022 d	4.818 b	4.420 b
	75	4.366 c	5.301 a	4.834 a
	150	4.885 b	4.659 bc	4.773 a
	Brassinolide			Average Brassinolide
Brassinolide × Seaweed Extract	0	3.961 c	4.850 ab	4.406 b
	0.75	4.590 b	4.984 a	4.787 a
	1.5	4.722	4.945 a	4.834 a
Average Seaweed extract		4.424 b	4.927 a	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

2_ Phosphorus content in leaves (%):

The results shown in Table (2) indicate that spraying with the growth regulator kinetin at a concentration of 150 mg L⁻¹ significantly increased the percentage of phosphorus in leaves, reaching 0.466%, compared to the other treatments. Spraying with the growth regulator brassinolide at a concentration of 0.75 mg L⁻¹ yielded the highest rate of 0.447% for the same trait, compared to the treatment using distilled water. However spraying with seaweed extract showed no statistically significant differences .

As for the two-way interaction between the growth regulator kinetin at a concentration of 150 mg L⁻¹ and the growth regulator brassinolide at a concentration of 0.75 mg L⁻¹, this had a significant effect on this trait, reaching 0.495%, compared to the other interactions. The interaction between the growth regulator kinetin at a concentration

of 150 mg L⁻¹ and 0 ml L⁻¹ of seaweed extract resulted in the highest value of 0.472% for the same trait, which did not differ significantly from the treatment with the interaction of 150 mg L⁻¹ kinetin and 3 ml L⁻¹ of seaweed extract, which reached 0.459% for this trait, compared to the other interactions. The interaction between the growth regulator Brassinolide at a concentration of 0.75 mg L⁻¹ and a concentration of 3 ml L⁻¹ of seaweed extract resulted in a 0.451% increase in this trait. As for the three-way interaction, we see that the spray treatment with concentrations of (150 mg L⁻¹ of kinetin + 0 mg L⁻¹ of brassinolide + 0 ml L⁻¹ of seaweed extract) gave a significant increase in this trait, reaching 0.552%, which did not differ significantly from the treatment with the interaction concentrations of (150 mg L⁻¹ of kinetin + 0.75 mg L⁻¹ of brassinolide + 3 ml L⁻¹ of seaweed extract), which reached 0.551% in the same trait compared to the other interactions

Table 2. Effect of spraying with Kinetin, Brassinolide, and Seaweed Extract and their interactions on the phosphorus content (%) in the leaves of young Grapevines cv. Halwani

Kinetin (mg L ⁻¹)	Brassinolide (mg L ⁻¹)	Seaweed Extract (ml L ⁻¹)		Kinetin × Brassinolide
		0	3	
0	0	0.319 h	0.458 b	0.389 d
	0.75	0.455 bc	0.349 gh	0.402 cd
	1.5	0.389 c-g	0.379 e-h	0.384 d
75	0	0.317 h	0.452 bc	0.384 d
	0.75	0.443 b-e	0.448 b-d	0.446 b
	1.5	0.410 b-f	0.344 gh	0.377 d
150	0	0.552 a	0.384 d-g	0.468 ab
	0.75	0.438 b-e	0.551 a	0.495 a
	1.5	0.426 b-e	0.441 b-e	0.434 bc
Kinetin				Average Kinetin
Kinetin × Seaweed Extract	0	0.388 b	0.395 b	0.392 b
	75	0.390 b	0.414 b	0.402 b
	150	0.472 a	0.459 a	0.466 a
Brassinolide				Average Brassinolide
Brassinolide × Seaweed Extract	0	0.396 bc	0.431 ab	0.414 b
	0.75	0.445 a	0.451 a	0.447 a
	1.5	0.408 bc	0.388 c	0.398 b
Average Seaweed extract		0.423 a	0.423 a	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

3_ Potassium content in leaves (%):

The results of the statistical analysis in Table (3) indicate that the percentage of potassium in the leaves increased significantly with the spray concentrations. The highest percentage of potassium was recorded when spraying with a concentration of 150 mg L⁻¹ of the growth regulator kinetin, reaching 1.037%, compared to the lowest percentage of 0.852% with the distilled water treatment. It was also shown that the increased effect of the growth regulator brassinolide significantly impacted the same trait, as the concentration of 0.75 mg L⁻¹ resulted in 1.041%, compared to the control treatment. However, spraying with seaweed extract showed no statistically significant differences. The dual interaction between spray treatments with the growth regulator

kinetin and brassinolide had a significant effect on this trait. The treatment with 150 mg L⁻¹ kinetin combined with 0.75 mg L⁻¹ brassinolide was the most effective, reaching 1.316%, compared to the other interactions. The dual interaction of 150 mg L⁻¹ kinetin combined with 0 ml L⁻¹ seaweed extract also had a significant effect, reaching 1.075%, compared to the other interactions. Finally, the dual interaction of 0.75 mg L⁻¹ brassinolide combined with 0 ml L⁻¹ seaweed extract yielded the highest potassium percentage value, reaching 1.105%, compared to the other interactions. As for the three-way interaction, we observed that spraying with concentrations of (150 mg L⁻¹ of kinetin + 0.75 mg L⁻¹ of brassinolide + 0 ml L⁻¹ of seaweed extract) yielded the highest value

for the same trait in the leaves, reaching 1.623%, compared to the control treatment.

Table 3. Effect of spraying with Kinetin, Brassinolide, and Seaweed Extract and their interactions on the potassium content (%) in the leaves of young Grapevines cv. Halwani

Kinetin (mg L ⁻¹)	Brassinolide (mg L ⁻¹)	Seaweed Extract (ml L ⁻¹)		Kinetin × Brassinolide
		0	3	
0	0	0.833 b	0.875 b	0.855 b
	0.75	0.828 b	0.911 b	0.869 b
	1.5	0.795 b	0.871 b	0.833 b
75	0	0.832 b	0.878 b	0.855 b
	0.75	0.864 b	1.011 b	0.938 b
	1.5	0.818 b	0.918 b	0.868 b
150	0	0.815 b	1.004 b	0.910 b
	0.75	1.623 a	1.009 b	1.316 a
	1.5	0.845 b	0.923 b	0.884 b
Kinetin				Average Kinetin
Kinetin × Seaweed Extract	0	0.819 b	0.886 b	0.852 b
	75	0.838 b	0.936 b	0.887 b
	150	1.075 a	0.979 ab	1.037 a
Brassinolide				Average Brassinolide
Brassinolide × Seaweed Extract	0	0.827 b	0.919 b	0.873 b
	0.75	1.105 a	0.977 ab	1.041 a
	1.5	0.819 b	0.904 b	0.862 b
Average Seaweed extract		0.934 a	0.934 a	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

4-Total Carbohydrate Content in Leaves

(%): The results shown in Table (4) indicate that spraying young Halwani grapevines with the growth regulator kinetin at a concentration of 150 mg L⁻¹ significantly affected the percentage of carbohydrates in the leaves, reaching 12.569%, an increase of 1.156% compared to the control treatment of 9.728%. Spraying with the growth regulator brassinolide at a concentration of 0.75 mg L⁻¹ yielded the highest percentage for this characteristic, reaching 11.982%, compared to the other concentrations. Meanwhile, spraying with seaweed extract at concentrations of (0 and 3) ml L⁻¹ resulted in no significant differences in this characteristic, reaching 10.879% and 11.033%, respectively. The two-way

interaction of the growth regulator kinetin at a concentration of 150 mg L⁻¹ with 1.5 mg L⁻¹ of brassinolide resulted in a significant increase in this trait of 13.019%, compared to the lowest concentration in the control treatment of 8.169%. Spraying with 150 mg L⁻¹ of kinetin in conjunction with spraying with 3 ml L⁻¹ of seaweed extract yielded the highest value for this trait at 13.768%, compared to the other interactions. The interaction of 0.75 mg L⁻¹ of brassinolide with 3 ml L⁻¹ of seaweed extract significantly affected this trait, with an increase of 12.324%, compared to the other interactions. The three-way interaction of the growth regulators kinetin and brassinolide, and seaweed extract at concentrations of (75 mg L⁻¹ of kinetin +

1.5 mg L⁻¹ of brassinolide + 0 ml L⁻¹ of seaweed extract) had a significant effect on this trait, reaching 14.907%. This did not differ significantly from the spray treatment at concentrations of (150 mg L⁻¹

of kinetin + 1.5 mg L⁻¹ of brassinolide + 3 ml L⁻¹ of seaweed extract), which reached 15.232%, compared to the other interactions.

Table 4. Effect of spraying with Kinetin, Brassinolide, and Seaweed Extract and their interactions on the carbohydrates content in the leaves (%) of young Grapevines cv.Halwani

Kinetin (mg L ⁻¹)	Brassinolide (mg L ⁻¹)	Seaweed Extract (ml L ⁻¹)		Kinetin × Brassinolide
		0	3	
0	0	7.547 fg	8.792 d-g	8.169 d
	0.75	12.488 a-e	9.909 b-g	11.198 a-c
	1.5	8.951 d-g	10.682 b-f	9.817 b-d
75	0	9.462 c-f	8.382 e-g	8.922 cd
	0.75	10.439 b-e	13.904 ab	12.172 ab
	1.5	14.907 a	6.318 g	10.612 a-d
150	0	11.318 a-f	12.913 a-d	12.115 ab
	0.75	11.990 a-e	13.160 a-c	12.575 ab
	1.5	10.806 b-f	15.232 a	13.019 a
Kinetin				Average Kinetin
Kinetin × Seaweed Extract	0	9.662 b	9.794 b	9.728 b
	75	11.603 b	9.535 b	10.569 b
	150	11.371 b	13.768 a	12.569 a
Brassinolide				Average Brassinolide
Brassinolide × Seaweed Extract	0	9.442 b	10.029 b	9.735 b
	0.75	11.639 ab	12.324 a	11.982 a
	1.5	11.555 ab	10.744 ab	11.149 ab
Average Seaweed extract		10.879 a	11.033 a	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

5-C/N ratio in leaves: The results of the statistical analysis in Table (5) show that the C/N ratio in leaves increased significantly when sprayed with the growth regulator kinetin at a concentration of 150 mg L⁻¹ giving the highest average C/N ratio in leaves, reaching 2.682, compared to the other treatments. As for the effect of spraying with the growth regulator brassinolide at concentrations of (0, 0.75 and 1.5) mg L⁻¹ no significant differences were observed between the means for this trait. However, spraying with seaweed extract showed no statistically significant differences. However, The two-way

interaction between the growth regulator kinetin at a concentration of 150 mg L⁻¹ and a concentration of 0.75 mg L⁻¹ of brassinolide resulted in a significant increase in the same trait, reaching 3.020, compared to the other interactions. While spraying with a concentration of 150 mg L⁻¹ of kinetin combined with a concentration of 3 ml L⁻¹ of seaweed extract resulted in a significant increase in the same trait, reaching 2.993. However, the binary interaction between the growth regulator brassinolide and seaweed extract showed no significant differences in this trait. The three-way interaction between the growth

regulators kinetin, brassinolide, and seaweed extract at concentrations of (75 mg L⁻¹ of kinetin + 1.5 mg L⁻¹ of brassinolide + 0 ml L⁻¹ of seaweed extract)

resulted in a significant effect on the same trait, reaching 3.376, compared to the other interactions.

Table 5. Effect of spraying with Kinetin, Brassinolide, and Seaweed Extract and their interactions on the C/N ratio in the leaves of young Grapevines cv. Halwani

Kinetin (mg L ⁻¹)	Brassinolide (mg L ⁻¹)	Seaweed Extract (ml L ⁻¹)		Kinetin × Brassinolide
		0	3	
0	0	2.199 b-g	1.891 d-g	2.045 b
	0.75	2.999 a-c	1.834 e-g	2.419 ab
	1.5	2.046 c-g	2.439 a-f	2.242 b
75	0	2.975 a-c	1.581 fg	2.278 b
	0.75	1.906 d-g	2.637 a-e	2.272 b
	1.5	3.376 a	1.179 g	2.277 b
150	0	2.183 b-g	2.833 a-e	2.508 ab
	0.75	2.906 a-d	3.124 ab	3.020 a
	1.5	2.024 c-g	3.010 a-c	2.517 ab
Kinetin				Average Kinetin
Kinetin × Seaweed Extract	0	2.414 bc	2.056 cd	2.235 b
	75	2.752 ab	1.799 d	2.276 b
	150	2.371 bc	2.993 a	2.682 a
Brassinolide				Average Brassinolide
Brassinolide × Seaweed Extract	0	2.452 a	2.102 a	2.277 a
	0.75	2.604 a	2.537 a	2.570 a
	1.5	2.482 a	2.209 a	2.346 a
Average Seaweed extract		2.513 a	2.283 a	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

6_ Protein content in leaves (%): The results of the statistical analysis shown in Table (6) indicate that the protein content in leaves increased significantly with the application of the growth regulator kinetin. The highest protein content was achieved with spraying at concentrations of (75 and 150) mg L⁻¹ reaching (30.210, 29.828)%, respectively, compared to the treatment with distilled water. Spraying with concentrations of (0.75 and 1.5) mg L⁻¹ of brassinolide also yielded the highest protein content, reaching 29.919% and 30.210%, respectively, compared to the control treatment. Spraying with a concentration of 3 ml L⁻¹ of seaweed extract resulted in a significant

improvement in this trait, reaching 30.791%, compared to the no-spray treatment. As for the two-way interaction between the growth regulator kinetin at a concentration of 75 mg L⁻¹ and a concentration of 0.75 mg L⁻¹ of brassinolide, the result was 33.474%, which showed a significant advantage in this trait compared to the other interactions. The two-way interaction treatment of 75 mg L⁻¹ of kinetin with a concentration of 3 ml L⁻¹ of seaweed extract was superior in this trait, reaching 33.134% compared to the other interactions. Meanwhile, the interaction treatment of (0.75 and 1.5) mg L⁻¹ of brassinolide with a concentration of 3 ml

L⁻¹ of seaweed extract recorded the highest percentage of superiority within the same trait, reaching (31.149 and 30.908) % compared to the other interactions. Regarding the three-way interaction, we observe the superiority of the interaction coefficients between the growth regulators

kinetin and brassinolide and the seaweed extract at concentrations of (0, 75 and 150) mg L⁻¹ of kinetin + (0, 0.75, 1.5) mg L⁻¹ of brassinolide + (0, 3) ml L⁻¹ of seaweed extract, reaching (33.688, 33.177, 33.616, 34.240, 33.391)% for the same trait, respectively.

Table 6. Effect of spraying with Kinetin, Brassinolide, and Seaweed Extract and their interactions on the protein content (%) in the leaves of young Grapevines cv. Halwani

Kinetin (mg L ⁻¹)	Brassinolide (mg L ⁻¹)	Seaweed Extract (ml L ⁻¹)		Kinetin × Brassinolide
		0	3	
0	0	21.865 e	29.135 b-d	25.500 e
	0.75	26.031 d	33.688 a	29.859 cd
	1.5	27.521 d	27.521 d	27.521 de
75	0	19.995 e	33.177 a	26.586 e
	0.75	34.240 a	32.708 ab	33.474 a
	1.5	27.625 d	33.516 a	30.570 bc
150	0	32.411 ab	28.630 cd	30.521 bc
	0.75	25.797 d	27.052 d	26.424 e
	1.5	33.391 a	31.688 a-c	32.539 ab
Kinetin				Average Kinetin
Kinetin × Seaweed Extract	0	25.139 d	30.115 b	27.627 b
	75	27.287 c	33.134 a	30.210 a
	150	30.533 b	29.123 bc	29.828 a
Brassinolide				Average Brassinolide
Brassinolide × Seaweed Extract	0	24.757 c	30.314 ab	27.536 b
	0.75	28.689 b	31.149 a	29.919 a
	1.5	29.512 ab	30.908 a	30.210 a
Average Seaweed extract		27.653 b	30.791 a	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

The increase in leaf content of total carbohydrates, protein, carbon-to-nitrogen (C/N) ratio, and macronutrients (N, P, K) can be explained by the physiological role of growth regulators in improving vital processes within the plant, Cytokinins contribute to delaying leaf senescence and increasing leaf area, leading to enhanced photosynthetic efficiency and increased carbohydrate accumulation in plant tissues [23]. These treatments also improve nutrient uptake and transport within the plant, particularly nitrogen, phosphorus, and potassium, resulting in increased leaf

content. This, in turn, positively impacts amino acid and protein synthesis and increases protein content [24]. Furthermore, an increase in carbohydrates relative to nitrogen leads to a higher carbon-to-nitrogen (C/N) ratio. This is attributed to the activation of photosynthesis and improved nutrient utilization efficiency within the plant, as well as the role of growth regulators in regulating physiological processes and the translocation of photosynthetic products within the plant. This, in turn, improves the plant's nutritional and physiological status

[25]. The increased nitrogen, phosphorus, potassium, carbohydrate, and protein content in leaves may be attributed to the physiological role of brassinolides in enhancing photosynthetic efficiency, increasing cell membrane permeability, and stimulating nutrient absorption and translocation within the plant. This leads to increased accumulation of organic compounds and minerals in plant tissues. Studies conducted on grapes have also indicated that spraying with brassinolides increases carbohydrate and soluble sugar content and improves the mineral content of leaves due to the activation of metabolic and physiological processes in the plant [26]. The increased protein content in leaves, as shown in Table (6), may be attributed to the role of brassinolide in increasing nitrogen uptake and stimulating the formation of amino acids and proteins, in addition to its effect on activating vital processes within cells. This leads to increased nitrogen metabolism and the accumulation of organic compounds in plant tissues [27]. The increased concentration of mineral elements when

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sprayed with the growth regulator brassinolide may be due to the activation of genes responsible for producing gibberellins in root tips, leading to increased root growth and consequently, increased mineral uptake [28]. The significant effect of seaweed extract may be attributed to its content of macro- and micro-minerals, amino acids, and growth regulators such as auxins, cytokinins, and gibberellins, which contribute to improved root growth and increased efficiency of nutrient uptake and translocation within the plant. Seaweed extracts also increase the efficiency of photosynthesis and the activity of the Rubisco enzyme, leading to increased synthesis and transport of photosynthetic products and carbohydrate accumulation within the leaves. Likewise, increased nitrogen uptake and activation of nitrogen metabolism processes contributed to increased formation and accumulation of amino acids and proteins in plant tissues, which had a positive effect on the chemical content of grapevine leaves [29, 30, 31].

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