



International Journal of Biological and Biomedical Research

Effect of Variety and Praying with Seaweed Extreac-Acdian and Salicylic Acid on Some Physicochemical Properties and Protein Pattern in the Leves of Grape Plant *Vitis Vinifera* L.

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Article Info

E-ISSN: 3107-7137

Volume: 02

Issue: 05

Received: 15-07-2026

Accepted: 13-08-2026

Published: 11-09-2026

Page No: 69-78

Abstract

This study took place in a private grove in Al-Nasr city, Thi-Qar Governorate, during the period from November 25, 2022 to August 1, 2023, on grape plants of the varieties Banki Al-Basra and Al-Halwani, which were 7 years old. This is to determine the effect of the variety and spraying with Acadian seaweed extract at concentrations of (0, 1, 3) g.L⁻¹ and salicylic acid at concentrations of (0, 50, 100) mg.L⁻¹ and their interactions on some leaf characteristics represented by leaf area, total soluble carbohydrates in the leaf, total chlorophyll pigment concentration, proline amino acid, and protein pattern. The results of the study showed an important effect of the variety factor on the studied attributes, as Banki Al-Basra variety significantly outperformed others in the amount of total soluble carbohydrates in the leaf. Halwani variety showed an important advantage in total chlorophyll concentration in leaf. The results also pointing that spraying grapevines with Acadian seaweed extract at a concentration of 1.3 g/L resulted in a significant increase in leaf area, total chlorophyll concentration, proline amino acid content, and total soluble carbohydrate content of the leaf compared to the control factor, The results also showed that spraying with salicylic acid at a concentration of (50-100) mg.L⁻¹ resulted in an obvious increase in leaf area, total soluble carbohydrates in the leaf, total chlorophyll pigment concentration, and the amino acid proline in the leaf. The coefficients of bilateral and trilateral overlaps were recorded a significant advantage over the control factor some of the studied attributes. The results of the protein pattern analysis of grapevine leaves within the experimental treatments showed differences between the treatments in the characteristics of protein bundles on polyacrylamide gel, which included the number, positions, and molecular weights of the bundles. The treatments differed in the number of protein bands, ranging from 3 bands in the interaction treatment between (Halwani variety and 0 g.L⁻¹ Acadian and 0 mg.L⁻¹ salicylic acid) to 6 bands in the two interaction treatments between Banki Al-Basra variety (0 g.L⁻¹ Acadian and 100 mg.L⁻¹ salicylic acid) and Halwani variety (3 g.L⁻¹ Acadian and 100mg.L⁻¹ salicylic acid). The molecular weights of these bands ranged from 7.500 kDa in the interaction treatment between (Basra Banki variety, 0 g.L-1 Acadian, and 50 mg.L⁻¹ salicylic acid) to 231.818 kDa in the interaction treatment between (Halwani variety, 3 g.L⁻¹ Acadian, and 50 mg.L⁻¹ salicylic acid).

DOI: <https://doi.org/10.54660/IJBBR.2026.2.5.69-78>

Keywords: Grape plant, Acadian seaweed extract, salicylic acid, leaf characteristics, protein type

Introduction

The grape (*Vitis vinifera* L.) is one of the most important fruits in temperate regions, belonging to *Vitaceae* family, which contain 14 genera and more than 1000 species ^[1] stated that grape cultivation in Mesopotamia dates to BC. The 2015 summer fruit production report indicates that grape production reached 93,629 tons for the 2015 summer season, an increase of 6.2% compared to the previous year's production of 57,994 tons. The average yield per tree was estimated at 26.8 kg for the year 2016, an

increase of 5.1% compared to the previous year, when it was estimated at (25.5 kg).

Grapes have great economic, nutritional and medical significance. They form an important part as human food and consuming them is necessary because they are rich in sugars, vitamins, organic acids, fats, proteins, fibers, minerals and calories [2]. Consuming grapes helps protect against heart, digestive, and urinary tract diseases. It inhibits the growth of free radicals in cells due to the presence of antioxidants such as carotene, anthocyanin, and phenolic compounds in the fruit [3-7]. Applying modern technologies in the service of agricultural orchard soils, such as plowing, hoeing, fertilizing, irrigation, etc., is a basic requirement to raise the efficiency of agricultural production. The foliar feeding method is one of the modern technologies to overcome the risks of environmental pollution. Seaweed extracts have been used as organic fertilizers to supplement, but not replace, basic fertilizers in agriculture [8]. These extracts work to increase and improve vegetative growth and yield in plants by increasing the efficiency of photosynthesis and respiration, and resistance to various environmental stresses such as salt stress and heat drought [9]. In addition to increasing the effectiveness of antioxidants through their role in the formation and preference of the activity of certain enzymes associated with resistance to biotic and abiotic stresses [10-12]. Many studies have been conducted on the effect of seaweed extracts on the growth and yield properties of fruit crops [13], noted that spraying grapevines of the Trakya Ilkeren variety with an extract of the seaweed *Ascophyllum nodosum* had positive effects. By spraying the leaves at concentrations of 1000, 3000, and 5000mg/L in three applications, the two concentrations (1000 and 3000 mg/L) showed a significant increase in the total carbohydrate content of the leaves compared to the control factor. It was found that treating grapevines of the Summer Royal variety with Acadian seaweed extract at a concentration of 1 g.L-1 led to a significant increase in the concentration of total chlorophyll pigment in the leaf compared to the control factor [14]. Salicylic acid is a plant hormone that plays an effective role in improving plant tolerance to various environmental and biological stresses, as it provides protection against salt and heat stresses resulting from heavy metals such as lead and mercury [15]. Moreover, this hormone regulates some physiological processes such as photosynthesis, flowering induction, ion absorption, hormonal balance, stomatal movement, and increased enzyme activity in the plant [16]. Many studies have addressed its positive effect on olive seedlings at a concentration of 200 mg. L-1 [17] and on bitter orange seedlings at concentrations of (100-200) mg. L-1 [18]. Given that the southern region of Iraq is subject to the harmful effects of environmental stress phenomena such as high salinity in irrigation water and grove soils, as well as the high temperatures during crop growth and ripening, which may reach 50°C at the peak of summer, Because grapevines are sensitive to such environmental stresses, this study was conducted to reduce these harmful effects and thus enable the expansion of this plant species in all orchards of the southern region of Iraq. The current study aims to determine the effect of spraying with Acadian seaweed extract and salicylic acid and their interactions on the properties of leaf area, carbohydrate quantity, chlorophyll pigment, proline amino acid, and protein pattern in the leaves of the two grape varieties, Banki Al-Basra and Al-Halwani.

Materials and Working Methods

Leaf Area (cm²)

Eight leaf samples were taken from each shrub, which represents an experimental unit, and the leaf area was measured separately using a Leaf area meter, then the average leaf area was extracted.

Determination of The Total Amount of Soluble Carbohydrates in The Leaves

This characteristic was assessed using the phenol-sulfuric acid modification colorimetric method (PSACM) [19]. The fourth leaf was removed from the apical meristem of each treatment replicate. After thorough cleaning, the leaves were put in an electric oven at 70°C for 48 hours. The leaves were then finely ground, and 0.5g of the dry, ground plant sample was taken from each treatment. It was placed in a test tube, 75ml of distilled water was added, the tube was sealed tightly, and it was heated in a water bath at 90°C for one hour, then cooled to room temperature and The solution was filtered through filter paper, and a volume of (5ml) of the filtrate was taken and (25 ml) of distilled water was added to it. Then (1ml) of this mixture was taken and (1ml) of phenol solution was added to it along with (5ml) of concentrated sulfuric acid and It was cooled to room temperature and the absorbance was measured using a spectrophotometer at a wavelength of 490 nanometers, and the total soluble carbohydrates were estimated using a standard curve in which glucose was used.

$$\text{Total soluble} = \frac{\text{Carbohydrate content (standard curve)} * \text{final extract} * \text{volume} * \text{dilution}}{\text{Sample weight mg/100g}}$$

Determination of Total Chlorophyll Pigment in Leaves

Total chlorophyll pigment in grapevine leaf samples was estimated by take 0.5 g of fresh leaves, and the pigments were extracted using 80% acetone [20]. The pigments were then estimated using a spectrophotometer; Chlorophyll (a) was measured at a wavelength of 663 nanometers and chlorophyll (b) at a wavelength of 645 nanometers. The total chlorophyll was estimated by calculating the concentration of the pigments as in the following equations:

$$\text{Total chlorophyll mg/L} = 20.2 \times \text{O.D. (645)} - 8.02 \times \text{OD (663)}$$

The results were then converted to units of (mg /100g).

Electrical Migration

The electrophoresis of proteins in dried grape leaves and for all treatments was carried out on polyacrylamide gel [21, 22]. The paper samples, cut into small pieces, were dried using the freeze-drying technique in a German-made Christ Alpha-2LD lyophilization machine for 72h until moisture was removed. They were then stored in airtight containers and kept in the freezer until use. Take 0.5g of each of the dry samples and grind them in a ceramic mortar with 1.5 ml of the prepared extraction solution at 4°C and place them in a refrigerated centrifuge at speed of 15,000 rpm for 30 min. Then take the filtrate and keep it in the refrigerator until the electrophoresis process is carried out. The migration was performed on polyacrylamide gel using a Vertical Slab Gel Apparatus (Cleaver Scientific, VK) in the presence of SDS denaturants [23]. The molecular weights of the protein bundles were estimated and plotted using Photocapt software.

Result

The results recorded in Table (1) illustrate the effect of the variety, Acadian seaweed extract, salicylic acid, and their interactions on the leaf area characteristic of the grape varieties Banki Al-Basra and Al-Halwani, no apparent differences were recorded between the two varieties in leaf area, but the highest increase in this characteristic was for the Basra Banki variety, reaching 103.90 cm².

Table (1) also shows that spraying grape with Acadian seaweed extract led to a significant characteristic for the two treatments (3,1) g.L-1 Acadian over the control treatment in leaf area per plant, which reached (113.70, 108.90) cm². In succession, while the control treatment plants gave the lowest leaf area per plant, which was (83.60) cm², and no significant differences were recorded between the two extract treatments in this trait.

The results in Table (1) pointing that spraying grapevines with salicylic acid resulted in the treatment with 100 mg/L salicylic acid significantly outperforming the other treatments in terms of leaf area per plant, reaching (112.30) cm². While the control treatment plants gave the lowest average leaf area per plant, at (91.60) cm², the treatment with 50 mg.L-1 salicylic acid significantly outperformed the control treatment plants in this trait (102.20) cm².

Regarding the effect of the dual interaction between the variety and the Acadian seaweed extract, the data in Table (1) indicate a significant superiority in the plants treated with the interaction (the Basra Banki variety and a concentration of 3 g/L Acadian), which reached (119.40) cm² which did not differ significantly from the interaction treatment (Basra

Banki variety and a concentration of 1 g/L Acadian) (112.10) cm², while the interaction treatment (Basra Banki and 0 g/L Acadian) gave the smallest leaf area (80.10) cm² which did not differ significantly from the interaction treatment (Halwani variety and 0 g.L-1 Acadian) (87.00) cm².

The dual interaction between the variety and salicylic acid also had a significant effect on leaf area in the plant (Table 1), as the interaction was superior (The Basra Banki variety and 100 mg.L-1 salicylic acid) significantly outperformed the other treatments in leaf area in the plant and recorded an average of (117.20) cm², while the treatment of the (Halwani variety and zero mg.L-1 salicylic acid) gave a leaf area in the plant of (91.00) cm².

The dual interaction between Acadian seaweed extract and salicylic acid (Table 1) showed a significant effect on leaf area of plant, the dual interaction (3 g.L-1 Acadian and 100 mg.L-1 salicylic acid) significantly outperformed the other interaction treatments in leaf area per plant (129.50) cm², while the control treatment for the interaction recorded the lowest leaf area per plant, which was 72.20 cm².

As for the tripartite interaction between the variety, Acadian seaweed extract, and salicylic acid, Table (1) shows a significant effect on leaf area in the plant, as the interaction (the Banki variety, 3 g.L-1 Acadian, and 100 mg.L-1 salicylic acid) significantly outperformed the other three interactions and recorded the highest leaf area of 139.36 cm², while the interaction between (Basra Banki variety, 0 g.L-1 Acadian, and 0 mg.L-1 salicylic acid) recorded the lowest leaf area of the plant, reaching 62.77 cm².

Table 1: Effect of variety, Acadian seaweed extract, salicylic acid and their interactions on leaf area (cm²) of the two grape varieties, Banki Al-Basra and Al-Halwani

Type overlap and Acadian extract	Salicylic acid (mg. L ⁻¹)			Acadian Extract (g.L-1)	Type	
	100	50	0			
80.10	89.50	88.10	62.77	0	Banki Al-Basra	
112.10	122.77	110.92	102.64	1		
119.40	139.36	107.43	111.47	3		
87.00	92.09	87.44	81.61	0	Al-Halwani	
105.60	110.07	111.10	95.64	1		
108.00	120.19	108.28	95.62	3		
	112.30	102.20	91.60	Average salicylic acid		
Average type	Interference of the type and salicylic acid					
103.90	117.20	102.20	92.30	Banki Al-Basra		
100.2	107.40	102.30	91.00	Al-Halwani		
Average extract of Acadian				overlap of Acadian extract and Salicylic acid		
83.60	90.80	87.80	72.20	0		
108.90	116.00	111.10	99.10	1		
113.70	129.50	107.90	103.50	3		
The smallest significant difference is at a probability level of 0.05.						
Type + Acadian + Salicylic acid	Acadian + Salicylic acid	Type + Salicylic acid	Type + Acadian	Salicylic acid	Acadian extract	Type
15.13	10.70	8.73	8.73	6.18	6.18	N.S.

Total chlorophyll pigment in leaves: The results recorded in Table (2) illustrate the influence of the variety, Acadian seaweed extract, salicylic acid, and the interactions between them on the chlorophyll pigment characteristic of the grape varieties Banki Al-Basra and Al-Halwani, the results in the table pointing that Halwani variety is significantly outperforms the Banki Al-Basra variety in the concentration of total chlorophyll pigment in the leaf, as it reached 38.40

mg/100 g fresh weight, while the concentration of this pigment in Halwani variety reached 32.91 mg/100 g fresh weight. This may be due to the genetic difference between the two varieties, which is reflected in the different concentration of this pigment in the leaves of the two grape varieties, Banki Al-Basra and Al-Halwani.

Regarding the effect of Acadian seaweed extract, the data in Table (2) show significant differences between the treatments

in the concentration of total chlorophyll pigment in the leaf, where the 3 g/L Acadian treatment significantly outperformed the other treatments in this characteristic, reaching 44.84 mg/100 g fresh weight, while the control treatment for the extract recorded the lowest concentration of this trait at 19.43 mg/100g (fresh weight), the 1 g/L treatment (Acadian) significantly outperformed the control plant treatment in the concentration of this pigment, reaching 37.09 mg/100g (fresh weight).

As for salicylic acid, Table (2) shows that the treatment with 100 mg/L of salicylic acid was significantly superior to the other acid treatments in terms of total chlorophyll pigment concentration in the leaf (44.84 mg/100 g fresh weight).

The results in Table (2) showed that the binary interaction between (Halwani variety and Acadian extract at a concentration of 3 g/L) significantly outperformed the other binary interactions in terms of total chlorophyll concentration in the leaf, recording (53.39) mg/100 g fresh weight. In contrast, the control treatment, the binary interaction between (the Banki Al-Basra variety and 3 g/L Acadian), recorded the lowest concentration of this pigment at 18.70 mg/100 g fresh weight.

The remaining binary interaction treatments, except for the interaction treatment between (Halwani variety and 03g.L-1Acadian), showed a significant superiority in this trait over the interaction treatment (Banki Al-Basra variety and 03g.L-1Acadian).

The interaction of the variety and salicylic acid also had a significant effect on the total chlorophyll pigment concentration in the leaf (Table 2). The interaction between (Halwani variety and salicylic acid at a concentration of 100 mg/L) significantly outperformed the other interactions in this trait, recording an average of (49.36 mg/100 g/fresh weight), while the interaction between (Halwani variety and salicylic acid concentration zero mg.100g-1) gave the lowest concentration of this pigment in the leaf, which was 22.33 mg.100g-1, which did not differ significantly from the interaction treatment (Banki Al-Basra variety and salicylic acid concentration zero mg.100g-1) (22.52) mg.100g-1 fresh weight.

As for the triple interactions, Table (2), the triple interaction between (Halwani variety, 3 g.L-1 Acadian extract, and 100 mg.L-1 salicylic acid) recorded the highest significant advantage in the concentration of total chlorophyll pigment in the leaf relative to the rest of the triple interaction treatments, as it reached 66.52 mg.100 g-1 fresh weight, while the triple interaction treatment between (the Banki variety, 0 g.L1- Acadian extract, and 0 mg.L1- salicylic acid) recorded the lowest concentration of this pigment in the leaf, which was 15.31 mg.100g1- fresh weight.

The effect of bilateral and trilateral interactions is attributed to the combined positive effect of the three factors mentioned above when discussing individual factors.

Table 2: Effect of variety, Acadian seaweed extract, salicylic acid and interactions between them on the concentration of total chlorophyll in the leaf (mg 0.100g 1-) for the two grape varieties Banki Al-Basra and Al-Halwani.

Type	Acadian Extract (g·L ⁻¹)	Salicylic Acid (mg·L ⁻¹)	Type × Acadian Extract	Type × Salicylic Acid	Acadian Extract × Salicylic Acid	Type × Acadian Extract × Salicylic Acid
Banki Al-Basra						
0	0	15.31	19.85	20.94	18.70	
1	1	26.47	31.22	39.91	32.53	
3	3	25.79	56.63	60.11	47.51	
Al-Halwani						
0	0	15.63	21.11	23.72	20.15	
1	1	22.58	44.52	57.84	41.65	
3	3	28.79	64.86	66.52	53.39	
Average Salicylic Acid	—	22.43	39.70	44.84	—	—
Average Type: Banki Al-Basra	—	22.52	35.90	40.32	32.91	—
Average Type: Al-Halwani	—	22.33	43.50	49.36	38.40	—
Average Acadian Extract: 0	0	15.47	20.48	22.33	19.43	—
Average Acadian Extract: 1	1	24.53	37.87	48.88	37.09	—
Average Acadian Extract: 3	3	27.29	60.74	63.31	44.84	—
LSD (P = 0.05): Type	1.79	—	—	—	—	—
LSD (P = 0.05): Acadian Extract	2.19	—	—	—	—	—
LSD (P = 0.05): Salicylic Acid	2.19	—	—	—	—	—
LSD (P = 0.05): Type + Acadian Extract	3.10	—	—	—	—	—
LSD (P = 0.05): Type + Salicylic Acid	3.10	—	—	—	—	—
LSD (P = 0.05): Acadian Extract + Salicylic Acid	3.79	—	—	—	—	—
LSD (P = 0.05): Type + Acadian Extract + Salicylic Acid	5.37	—	—	—	—	—

Total Amount of Soluble Carbohydrates in The Leaves

The results recorded in Table (3) show the effect of the variety, Acadian seaweed extract and salicylic acid on the amount of total soluble carbohydrates in the leaf of the grape varieties Banki Al-Basra and Al-Halwani. The results indicate significant differences between the two grape varieties, Banki Al-Basra and Halwani, in the amount of total soluble carbohydrates in the leaf. The Banki Al-Basra variety significantly outperformed Halwani variety in this

characteristic (11.71 mg/100g), while Halwani variety recorded (11.30 mg/100g).

This difference in the total amount of soluble carbohydrates in the leaf for both varieties may be attributed to the nature of the photosynthesis process in producing food collected in the leaves, which is related to the genetic factors specific to the variety, and whose effect is reflected in increasing the total amount of soluble carbohydrates in the leaf.

The same table as shown the Acadian seaweed extract, at a

concentration of 3 g/L, significantly outperformed the other treatments in the amount of total soluble carbohydrates in the leaf, reaching 12.35 mg/100 g, while the control treatment for the extract recorded the lowest average for this characteristic, reaching 10.48 mg/100 g.

As for the effect of salicylic acid, the treatment with 100 mg.L⁻¹ salicylic acid significantly outperformed the other acid treatments in the amount of total soluble carbohydrates in the leaf, reaching 12.35 mg/100g, while the acid control treatment gave the lowest average for this advantage, reaching 10.45 mg/100g.

The increased total carbohydrate content in grape leaves, resulting from higher salicylic acid concentration, could be attributed to its role in maintaining chlorophyll molecules in a healthy and functional state by preserving the fine structure of chloroplasts, particularly the thylakoid membranes which positively affects the increase in the efficiency of the photosynthesis process and activates the role of the enzymes involved in this process, which contributes to increasing the production of total soluble carbohydrates, the accumulation of which increases in the leaves of grape plants sprayed with salicylic acid [35-37].

The results in Table (3) show that the binary interaction between (the Basra Banki variety and 3 g. L1- Acadian extract) significantly outperformed the other extract interactions in the amount of total soluble carbohydrates in the leaf and recorded an average of 12.52 mg.100 g⁻¹, while the interaction between (Halwani variety and 0 g. L1- Acadian extract) recorded the lowest average for this trait, which was 10.11 mg.100 g⁻¹.

The results in Table (3) also indicate that the binary interaction between (the Basra Banki variety and salicylic acid at a concentration of 100 mg.L⁻¹) was significantly superior to the rest of the interaction treatments in the amount of total soluble carbohydrates in the leaf, as it reached 12.33 mg.100g⁻¹, while the interaction between (Halwani variety and zero mg.L⁻¹ salicylic acid) recorded the lowest average for this trait, which reached 10.09 mg.100g⁻¹. As for the binary interaction between Acadian extract and salicylic acid (Table 3), the interaction between (Acadian extract 3 g. L1- and salicylic acid concentration 100 mg. L1-) significantly outperformed the other interactions in the total amount of soluble carbohydrates in the leaf, reaching 13.28 mg. L1-, while the control treatment for the interaction gave the lowest average for this trait, reaching 9.79 mg. 100 g⁻¹.

Table (3) shows significant differences between the three interactions in the total amount of soluble carbohydrates in the leaf, as the three interactions were recorded between (Basra Banki variety, 3g.L1- Acadian extract, and 100mg.L1- salicylic acid) showed the highest significant increase in total soluble carbohydrates in the leaf compared to the other triple interactions, reaching (13.35) mg/100 g/L, while the triple interaction between (Halwani variety and 0 g. L1-Acadian extract and 0 mg. L1-) recorded the lowest average for this trait, which was 9.21 mg.100 g⁻¹.

The effect of the two-way and three-way interactions is attributed to the positive effect of the three study factors on the total amount of soluble carbohydrates in the leaves of the two varieties, Banki Al-Basra and Al-Halwani.

Table 3: Effect of variety, Acadian seaweed extract, salicylic acid and interactions between them on the total amount of soluble carbohydrates in the leaf (mg.100g⁻¹) for the two grape varieties, Banki Al-Basra and Al-Halwani

Type	Acadian Extract (g·L ⁻¹)	Salicylic Acid (mg·L ⁻¹)	Type × Acadian Extract	Type × Salicylic Acid	Acadian Extract × Salicylic Acid	Type × Acadian Extract × Salicylic Acid
Banki Al-Basra						
0	0	10.36	10.87	11.29	10.84	
1	1	11.24	11.67	12.35	11.76	
3	3	11.35	12.86	13.35	12.52	
Al-Halwani						
0	0	9.21	10.43	10.71	10.11	
1	1	10.61	11.73	12.49	11.61	
3	3	10.45	12.88	13.20	12.18	
Average Salicylic Acid		10.45	11.74	12.35		
Average Type – Banki Al-Basra		10.98	11.80	12.33	11.71	
Average Type – Al-Halwani		10.09	11.68	12.14	11.30	
Average Acadian Extract – 0	0	9.79	10.65	11.00	10.48	
Average Acadian Extract – 1	1	10.93	11.70	12.42	11.68	
Average Acadian Extract – 3	3	10.90	12.87	13.28	12.35	
LSD (P = 0.05) – Type	0.14					
LSD (P = 0.05) – Acadian Extract	0.17					
LSD (P = 0.05) – Salicylic Acid	0.17					
LSD (P = 0.05) – Type + Acadian Extract	0.25					
LSD (P = 0.05) – Type + Salicylic Acid	0.25					
LSD (P = 0.05) – Acadian Extract + Salicylic Acid	0.30					
LSD (P = 0.05) – Type + Acadian Extract + Salicylic Acid	0.43					

Proline's Concentration in Leaves

The measurements recorded in Table (4) explain the effect of the variety, Acadian seaweed extract, salicylic acid, and their interactions on the leaf concentration of proline in the grape varieties Banki Al-Basra and Al-Halwani, the results recorded in the table indicates the effects of the variety,

Acadian seaweed extract, and salicylic acid on the concentration of the amino acid proline in the leaves of the grape varieties Banki Al-Basra and Al-Halwani. The table data showed that there are no significant differences between the two varieties in the concentration of the amino acid proline.

As for the effect of Acadian seaweed extract, the data in Table (4) showed significant differences between the extract treatments, with the 3 g L⁻¹ treatment significantly exceeding all extract treatments in concentration of the amino acid proline, reaching 3.87 mg/100 g, while the control plants gave the lowest proline concentration (1.16 mg/100 g), the Acadian treatment (1 g/L) significantly outperformed the control treatment in this trait (2.68 mg/100 g), for example, it protects against heat stress because it is one of the amino acids involved in building proteins and preventing their breakdown or lysis within cells.

Regarding the effect of salicylic acid levels on the concentration of the amino acid proline in the leaf, Table (4) shows that the treatment with 100 mg/L of salicylic acid significantly exceeded the other treatments in proline concentration, which reached (3.30 mg/100 g), while the control treatment plants gave the lowest average leaf proline concentration (1.53 mg/100 g), the 50 mg L⁻¹-salicylic acid treatment significantly superiority the control treatment in this trait (2.88 mg/100 g).

As for the effect of the binary interaction between the variety and the concentration of Acadian, the data in Table (4) showed a significant superiority in plants treated between (Al-Halwani variety and 3 g.L⁻¹ Acadian), which reached 4.08 mg/100 g, which differed significantly from the rest of the interaction treatments in this trait. The interaction treatment (Banki Al-Basra and 0 g L⁻¹ Acadian) gave the lowest concentration of proline in the leaf 1.12 mg/100 g. Regarding the bilateral interaction between the variety and salicylic acid, the data in Table (4) show a significant superiority in the plants treated with the interaction between (Halwani variety and 100 mg.L⁻¹ salicylic acid), which amounted to 3.40 mg/100 g), which did not differ

significantly from the treatment with the interaction (the Banki Al-Basra variety and a concentration of 100 mg.L salicylic acid) which amounted to (3.20 mg /100 g) relative to the rest of the interaction treatments, while the interaction treatment between (Halwani variety and zero mg 0L⁻¹ salicylic acid) recorded (1.46 mg /100 g) the lowest concentration of proline in the leaf.

Furthermore, significant differences were recorded in the binary interaction coefficients between Acadian seaweed extract and salicylic acid, as can be seen from Table (4). The treatment (3 g.L⁻¹ Acadian and 100 mg.L⁻¹ salicylic acid) significantly outperformed all other interaction coefficients in the concentration of the amino acid proline. However, it did not differ significantly from the interaction treatment (3 g.L⁻¹ Acadian and 50 mg.L⁻¹ salicylic acid) in this trait, which amounted to (4.68) mg.100 g⁻¹, while the control treatment gave the lowest concentration of proline in the leaf, which amounted to (0.84 mg/100 g).

As for the effect of the triple interaction between the variety, Acadian extract and salicylic acid, the data in Table (4) show significant differences between the treatments in the concentration of the amino acid proline in the leaf, as the interaction treatment between (Halwani variety, 3 g.L⁻¹ Acadian and 100 mg.L⁻¹ salicylic acid) significantly outperformed all other interaction treatments and recorded 5.33 mg/100 g, while the interaction treatment between (Halwani variety and 0 g.L⁻¹ Acadian and 0 mg.L⁻¹ salicylic acid) gave the lowest concentration of proline in the leaf 0.82 mg/100 g. The effects of the two and three interactions on the concentration of the amino acid proline in the leaves of the grape plant are referring to the combined effect of the study factors when discussed individually.

Table 4: Effect of the variety, Acadian seaweed extract, salicylic acid and the interactions between them on the concentration of the amino acid proline in the leaf (mg.100g⁻¹) of the two grape varieties, Banki Al-Basra and Al-Halwani.

Type	Acadian Extract (g.L ⁻¹)	Salicylic Acid (mg.L ⁻¹)	Type × Acadian Extract	Type × Salicylic Acid	Acadian Extract × Salicylic Acid	Type × Acadian Extract × Salicylic Acid
Banki Al-Basra						
0	0	0.86	0.95	1.55	1.12	
1	1	1.77	3.04	3.93	2.92	
3	3	2.14	4.70	4.13	3.66	
Al-Halwani						
0	0	0.82	1.07	1.70	1.20	
1	1	1.29	2.88	3.17	2.45	
3	3	2.26	4.66	5.33	4.08	
Average Salicylic Acid		1.53	2.88	3.30		
Average Type – Banki Al-Basra		1.59	2.90	3.20	2.57	
Average Type – Al-Halwani		1.46	2.87	3.40	2.58	
Average Acadian Extract – 0	0	0.84	1.01	1.63	1.16	
Average Acadian Extract – 1	1	1.54	2.97	3.55	2.68	
Average Acadian Extract – 3	3	2.20	4.68	4.73	3.87	
LSD (P = 0.05) – Type	N.S.					
LSD (P = 0.05) – Acadian Extract	0.29					
LSD (P = 0.05) – Salicylic Acid	0.29					
LSD (P = 0.05) – Type + Acadian Extract	0.42					
LSD (P = 0.05) – Type + Salicylic Acid	0.42					
LSD (P = 0.05) – Acadian Extract + Salicylic Acid	0.52					
LSD (P = 0.05) – Type + Acadian Extract + Salicylic Acid	0.73					

Electrical Migration

Table (5) shows the results of the protein profile of the grape leaves studied, the effect of the variety, Acadian seaweed extract, salicylic acid, and their interaction in the gel electrophoresis process, it is observed that there are

differences between the treatments in the protein bundles on the acrylamide gel in terms of the size, distance, height of these bundles and their molecular weights, and the number of molecular bundles ranges between (6 - 3) bundles depending on the treatment 8 yielded the fewest protein bundles (3),

while treatments (11, 12, 14, 16) yielded 4 protein bundles, and treatments (9, 10, 13, 15, 7) yielded 5 protein bundles. Treatments 17 and 18, however, recorded 6 protein bands on polyacrylamide gel. As for the protein bundle positions and their molecular weights on polyacrylamide gel, Table (5), some treatments had the same first bundle position and the same molecular weight. This is observed in treatments 8, 7, 6, 5, 4, 3, 2 (80.732 kDa), treatments 10, 9 (187.748 kDa), and treatments 13, 12, 11 (194.961 kDa), Treatments 16 and 15 (208.822 kDa) varied, while the remaining treatments differed in their effect on the molecular weight of the first protein bundle, which reached (231.818, 225.000, 202.332 kDa) in treatments 19, 18, and 14, respectively.

As for the third protein bundle, some treatments had the same bundle position and the same molecular weight, such as treatments 7, 2 (124.535 kDa), treatments 6,5,4,3 (122.101 kDa), treatments 11,10,9 (129.488 kDa), and treatments 15,14,13,12 (132.000 kDa), Treatments 16, 17, and 18 had a molecular weight of 134.535 kDa, while treatments 19 and 8 varied in their effect on the molecular weight of the second protein bundle, which reached (137.086, 126.999 kDa)

respectively. As for the third protein bundle, the treatments varied in the molecular weight of this bundle, except for treatments 11 and 7, which recorded the lowest molecular weight of the bundle (44.822 kDa), while treatment 8 recorded the highest molecular weight of the bundle (90.270 kDa).

As for the effect of the treatments on the fourth protein bundle, it was also varied. Treatment 7 recorded the lowest molecular weight for this bundle, which was (34.061 kDa), while treatments 14 and 5 shared the highest molecular weight for the fourth protein bundle, which was (46.645 kDa). As for the effect of the treatments on the molecular weight of the fifth protein bundle, it varied in all treatments. Treatment 7 recorded the lowest molecular weight of the fifth protein bundle, which was (7.500 kDa), while treatment 5 recorded the highest molecular weight of the fifth protein bundle, which was (39.487 kDa). Treatments 18 and 17 also recorded variations in the molecular weight of the sixth protein bundle, ranging between (28.813 and 12.669 kDa) respectively.

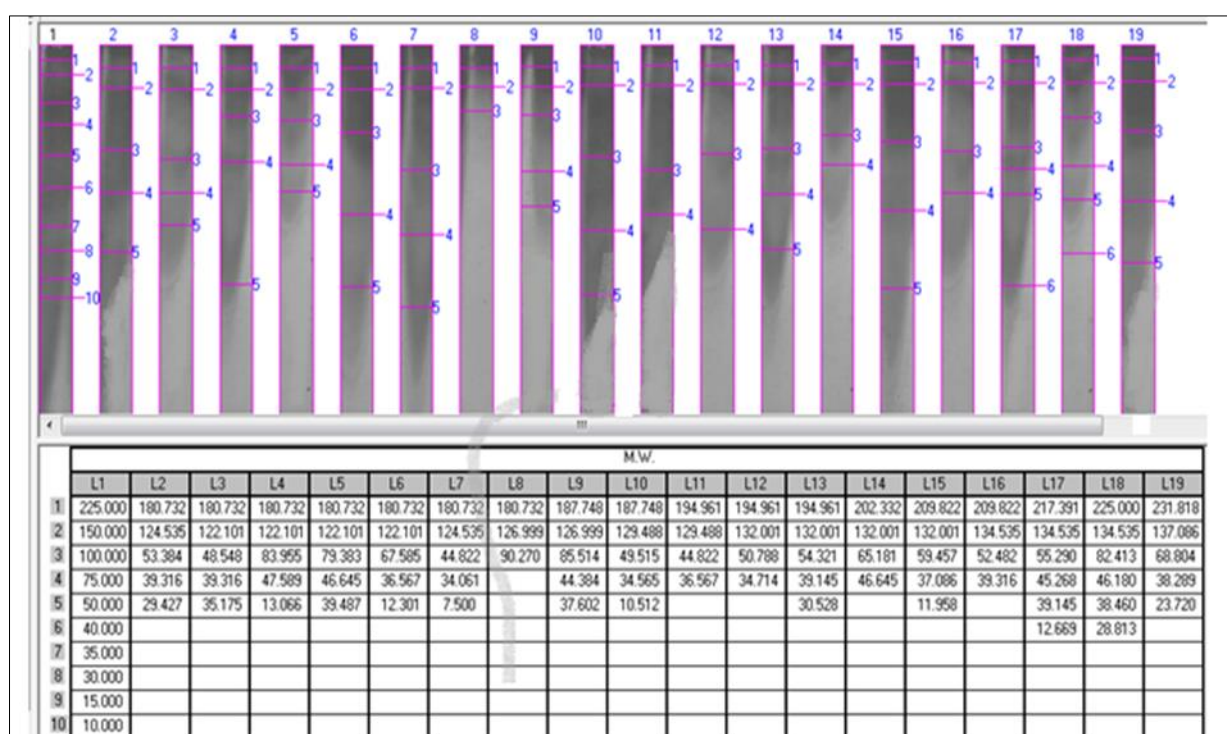


Fig 1: Shows the area, height, molecular weight, and protein band size for all study treatments

Discussion

The results recorded in Table (1) illustrate the effect of the variety, Acadian seaweed extract, salicylic acid, and their interactions on the leaf area characteristic of the grape varieties Banki Al-Basra and Al-Halwani. The reason for the increased leaf area of the grape plant due to the increased concentration of Acadian seaweed extract may be due to the role of the components of this extract in maintaining the integrity of plasma membranes against various environmental stressors. This increases the flow of water to the leaf cells, as well as the transfer of nutrients and growth hormones, which activate cell division and cell elongation processes, thus increasing the leaf area of the grape plant.

The significant increase in leaf area of grapevines sprayed with this acid may be attributed to its role in enhancing the efficiency of photosynthesis and food production in the

leaves, as well as the accumulation of its byproducts, such as carbohydrates, in plant tissues this includes the leaves, and then the connection of these materials to cell division and cell elongation processes, which lead to stimulating growth and increasing the leaf area in these plants.

The effect of bilateral and trilateral interactions may be attributed to the combined positive effect of the three factors mentioned above when discussing individual factors (23). These results are consistent with many studies conducted on Halwani Lebanon and Kamali grape varieties (23). It is also consistent with the results obtained through several studies on types of strawberry plants that were sprayed with extracts from different types of seaweed (24-27).

The results recorded in Table (2) illustrate the influence of the variety, Acadian seaweed extract, salicylic acid, and the interactions between them on the chlorophyll pigment

characteristic of the grape varieties Banki Al-Basra and Al-Halwani. The increase in total chlorophyll concentration in grape leaves may be attributed to the role of the extract's components in the process of building and maintaining these pigments in the leaves, which depends on providing essential elements such as nitrogen, phosphorus, potassium, and iron help build this pigment by building proteins associated with the chlorophyll pigment and its enzymes, thus protecting the pigment molecules and preventing them from being damaged (28-30).

These results are similar to those obtained by several studies on apples and strawberry plants, where it was shown that spraying these plants with seaweed extracts plays a role in increasing the total chlorophyll pigment in the leaves this is achieved by increasing the concentration of NPK elements present in these extracts and the effect of these elements on the biosynthetic process (10, 25, 26).

Nitrogen plays a major role in building the porphyrin ring that is part of the chlorophyll molecule and phosphorus play an auxiliary role in the formation of important proteins in the process of building chloroplasts, in addition to the role of potassium which contributes to the activation of many enzymes that carry out the processes of forming chloroplasts (31, 32).

The increase in the concentration of total chlorophyll pigment in the leaf may be attributed to the role of salicylic acid in regulating physiological processes in the plant, including the entry and transfer of ions within cells and its contribution to increasing pigment synthesis, and thus the strength and cohesion of chloroplast proteins (33, 34). The effect of bilateral and trilateral interactions is attributed to the combined positive effect of the three factors mentioned above when discussing individual factors.

The results recorded in Table (3) show the effect of the variety, Acadian seaweed extract and salicylic acid on the amount of total soluble carbohydrates in the leaf of the grape varieties Banki Al-Basra and Al-Halwani. This difference in the total amount of soluble carbohydrates in the leaf for both varieties may be attributed to the nature of the photosynthesis process in producing food collected in the leaves, which is related to the genetic factors specific to the variety, and whose effect is reflected in increasing the total amount of soluble carbohydrates in the leaf.

The increase in the total amount of soluble carbohydrates of the grape plant when the concentration of Acadian extract is increased may be due to the role of the components of this extract in the biosynthesis processes of carbohydrates, which results in an increase in their accumulation in the leaves (13, 35, 36).

The increased total carbohydrate content in grape leaves, resulting from higher salicylic acid concentration, could be attributed to its role in maintaining chlorophyll molecules in a healthy and functional state by preserving the fine structure of chloroplasts, particularly the thylakoid membranes which positively affects the increase in the efficiency of the photosynthesis process and activates the role of the enzymes involved in this process, which contributes to increasing the production of total soluble carbohydrates, the accumulation of which increases in the leaves of grape plants sprayed with salicylic acid (35, 37).

The effect of the two-way and three-way interactions is attributed to the positive effect of the three study factors on the total amount of soluble carbohydrates in the leaves of the two varieties, Banki Al-Basra and Al-Halwani.

The measurements recorded in Table (4) explain the effect of the variety, Acadian seaweed extract, salicylic acid, and their interactions on the leaf concentration of proline in the grape varieties Banki Al-Basra and Al-Halwani, the results recorded in the table indicates the effects of the variety, Acadian seaweed extract, and salicylic acid on the concentration of the amino acid proline in the leaves of the grape varieties Banki Al-Basra and Al-Halwani. The table data showed that there are no significant differences between the two varieties in the concentration of the amino acid proline.

As for the effect of Acadian seaweed extract, the data in Table (4) showed significant differences between the extract treatments, with the 3 g L⁻¹ treatment significantly exceeding all extract treatments in concentration of the amino acid proline, reaching 3.87 mg/100 g, while the control plants gave the lowest proline concentration (1.16 mg/100 g), the Acadian treatment (1 g/L) significantly outperformed the control treatment in this trait (2.68 mg/100 g), for example, it protects against heat stress because it is one of the amino acids involved in building proteins and preventing their breakdown or lysis within cells. This is reflected positively in protecting grape plants from heat stress during the growing season by amend the osmotic potential of plant cells and maintaining the structure of their cell membranes (38- 40).

The increased concentration of this amino acid in grape leaves may be due to salicylic acid's role in supporting plant tolerance to environmental exertion such as heat exertion, which occurs at the peak of summer when temperatures reach over 40°C. This is achieved through the synthesis of the amino acid proline in the cytoplasm or chloroplasts and its increased accumulation in plant tissues such as leaves, in order to strengthen the defense system of grapevines against these stresses, since salicylic acid protects plasma membranes from oxidation and regulates osmosis and water flow into cells this provides adequate protection for cell organelles to perform their physiological functions with high efficiency, which is reflected in improved vegetative, fruit, and root growth characteristics of grape plants (41), namely that increased leaf proline content is an important indicator of plant resilience to various stress conditions.

Table (5) shows the results of the protein profile of the grape leaves studied, the effect of the variety, Acadian seaweed extract, salicylic acid, and their interaction in the gel electrophoresis process, This variation in the number of protein bands on the polyacrylamide gel may be attributed to the interaction between the study factors the cultivar, the Acadian seaweed extract, and salicylic acid in the signaling and regulation process leading to gene expression. Thus, the synthesis of new types of proteins necessary for plants to withstand different environmental stress conditions, and all of this is subject to genetic action and control according to the influencing factors (41). These results indicate that there is a clear effect of the combined interaction between the study factors in the similarity and difference sites in the number of protein bundles and their molecular weights on the polyacrylamide gel of the treatments, which indicates their effect on the subtle characteristics and changes they produced in grape plants, including the protein pattern characteristics in the leaves. In study of the protein pattern of the leaves of 6 and 13 agricultural varieties of grapes respectively, it's found a great variation in the number of protein bundles and the molecular weights in the leaves of these varieties (42, 43).

Note That

Transaction 1 represents the standard transaction

Treatment 2 represents Halwani variety, 3 g Acadian, and 0 mg/L Salicylic Acid.

Treatment 3 represents the Basra Banki variety, 1 g Acadian, and 0 mg/L Salicylic Acid.

Treatment 4 represents Halwani variety, 1 g of Acadian, and 0 mg/L of Salicylic Acid.

Treatment 5 represents the Banki Al-Basra variety, 3 g of Acadian, and 100 mg/L of Salicylic Acid.

Treatment 6 represents the Banki Al-Basra variety, 3 g of Acadian, and 0 mg/L of Salicylic Acid.

Treatment 7 represents Halwani variety, 0 g Acadian, and 50 mg/L Salicylic Acid.

Treatment 8 represents Halwani variety, 0 g Acadian, and 0 mg/L Salicylic Acid.

Treatment 9 represents Halwani variety, 1 g Acadian, and 50 mg/L Salicylic Acid.

Treatment 10 represents the Basra Banki variety, 1 g of Acadian, and 100 mg/L of Salicylic Acid.

Treatment 11 represents Halwani variety, 1 g of Acadian, and 100 mg/L of Salicylic Acid.

Treatment 12 represents the Basra Banki variety, 0 g of Acadian, and 0 mg/L of Salicylic Acid

Treatment 13 represents the Basra Banki variety, 3 g Acadian, and 50 mg/L Salicylic Acid.

Treatment 14 represents the Basra Banki variety, 0 g Acadian, and 50 mg/L Salicylic Acid.

Treatment 15 represents the Basra Banki variety, 1 g Acadian, and 50 mg/L Salicylic Acid.

Treatment 16 represents Halwani variety, 0 g Acadian, and 100 mg/L Salicylic Acid.

Treatment 17 represents the Banki Al-Basra variety, 0 g Acadian, and 100 mg/L Salicylic Acid.

Treatment 18 represents Halwani variety, 3 g Acadian, and 100 mg/L Salicylic Acid.

Treatment 19 represents Halwani variety, 3 g Acadian, and 50 mg/L Salicylic Acid.

The Description

- Grapevines are crucial crops that is affected by environmental pressures such as heat and salinity.
- Previous research has indicated that seaweed extracts and salicylic acid can promote growth and stress tolerance.
- A field experiment was conducted in Dhi Qar, Iraq, tested two grapevine varieties with different concentrations of seaweed extract and salicylic acid. The results showed significant improvement in leaf area, chlorophyll content, soluble carbohydrates, and proline levels, particularly with the use of a mixture of 3 g/L seaweed extract and 100 mg/L salicylic acid.

The study concludes that this integration enhances grapevine stress tolerance and supports sustainable agricultural practices, offering strategies for better grape production under stress.

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How to Cite This

Al-Saidy SJ. Effect of variety and praying with seaweed extract-Acdian and salicylic acid on some physicochemical properties and protein pattern in the leaves of grape plant *Vitis vinifera* L. *Int J Biol Biomed Res.* 2026;2(5):69-78. doi:10.54660/IJBBR.2026.2.5.69-78.

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