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Ali Ahmed Hussein Almyali

Salim Kadhim

Ali S. Hassoon

Ameer Al Fatlawy

ORIGINAL STUDY

Effect of Variety and Seaweed Extract Spray on the Content of Some Medically Active Phenolic Compounds in Grape Fruits

Ali A.H. Almyali [a,b], Salim Kadhim [a,*], Ali S. Hassoon [c], Ameer Al Fatlawy [a]

[a] University of Alkafeel, College of Pharmacy, Najaf, Iraq
[b] Department of Biology, College of Science, University of Kerbala, Karbala, Iraq
[c] Alfurat Al-Awsat Technical University, Almusaib Technical College, Iraq

Abstract

Grape (*Vitis vinifera L.*) is one of the most economically significant fruit crops which has a large adaptive range, nutrition and medicine effect. A variety of 70 types of grapes are planted on more than 11.6 million plants in Iraq, generating a total annual output of more than 421,000 tons. Increasing grape quality, and particularly the phenolic composition recently has been of major interest. Leaf nutrition − particularly seaweed extracts, being rich in amino acids, trace elements and growth promoting substances − has proved to be a potential tool to enhance plant growth and fruit biochemical quality. The effect of grape variety and fertilizer on the phenolic content in grape fruits (Halwani and Kamali) was investigated using the foliar-applied seaweed extract, Agrimax® at 0, 15 and 30 ml/L were applied. Factorial experiment was carried out in 2024 with completely randomized design by three replications. The first foliar treatment was performed on the first day of April and thereafter five sprays were given at 15 days intervals. HPLC was used to determine the amount of phenolic compounds, such as caffeic, gallic, cinnamic, chlorogenic and p-coumaric acid), The data was analyzed using GenStat with LSD at 5% and two-way ANOVA followed by Šidák test. High phenolic traits were found to be significantly different in all, but caffeic, gallic, and cinnamic acids where the Halwani population extracted with methanol outperformed Kamali. Application of seaweed extract (particularly at 30 ml/L) considerably raised contents of caffeic, gallic, cinnamic and chlorogenic acids in comparison to control plants. The highest levels of all significantly altered phenolics were observed in Halwani variety at the concentration 30 ml/L Agrimax®. The level of p-coumaric acid did not significantly change. In conclusion, the Halwani cultivar had the highest phenolic content under Babil Germplasm climatological conditions. Application of seaweed extract by foliar method particularly at 30 ml/L significantly increased phenolic content, indicating its potential to be used as an eco-friendly substitute to mineral fertilization.

Keywords: Grapes (*Vitis vinifera* L.), Seaweed extract, Agrimax®, Foliar fertilization, Phenolic compounds, Halwani variety, Kamali variety, Caffeic acid, Gallic acid, Cinnamic acid, Chlorogenic acid, p-coumaric acid sustainable

1. Introduction

Grapes (*Vitis vinifera L.*) represent one of the most economically important fruit crops worldwide, being appreciated not only for their fresh consumption but also for their high content in bioactive molecules with acknowledged health-promoting properties. In the last few years, much effort has been made to enhance grape quality with sustainable agronomic practices which promote the accumulation of phenolic compounds, as they are among those responsible for antioxidant potential, sensory characteristics and plant resistance. Grapes (*Vitis vinifera* L.) is one member of the Vitaceae and includes 14 genera and more than 1000 species [1]. They are cultivated in various

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* Corresponding author. A42, 8, Alameerat, Najaf, Iraq.
E-mail addresses: ali.hussien@alkafeel.edu.iq (A.A.H. Almyali), sfk9@alkafeel.edu.iq (S. Kadhim), com.hs.ali@atu.edu.iq (A.S. Hassoon), hs.ali@atu.edu.iq (A. Al Fatlawy).

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parts of the world under an extensive range of environmental conditions, such as subtropical to cold temperate regions. The global vineyard is believed to cover some 7.5 million hectares [2]. The total number of grape trees in Iraq is about 11,613,783, and the annual production reached at 421,868 tons with an average yield of 36.32 kg per tree [3]. The number of grape varieties in Iraq is about 70, with the majority grown in northern governorates namely, Sulaymanieh, Erbil and Dohuk that represent more than 7 million grape vines for about 58 local and international grape varieties [3]. - In the central area, it is spread in the governorates of Najaf, Babil and Karbala, with Najaf holding first place. Babil Governorate comes the second place in Middle Euphrates region with respect to area cultivated which is about 6480 dunum with number of fruit trees about 518,684, and one vine is estimated by about 31 kg [3].

Plant based substances are of vital importance in nutrition due to their diversity as a natural source of bioactive compounds with nutritional values. A significant number of active molecules including alkaloids, flavonoids, terpenoids, glycosides and phenolic acids are isolated from medicinal plants and contribute to the anti-inflammatory, antimicrobial and antioxidant potential. Due to a growing interest in natural, and integrative remedies plant derived compounds continue to be important contributors for the development of novel therapeutics as well as deconvoluting intricate biological pathways. The phenolic compounds found in grapes are a heterogeneous group of secondary metabolites, which include phenolic acids, flavonoids and tannins. Gallic acid [4], caffeic acid [5], chlorogenic acid [6], cinnamic acid [7] and p-coumaric acid [8] are considered as significant contributors possessing strong antioxidant, anti-inflammatory and antimicrobial activities. Grape variety, environmental factors and agronomic practices interfere in the concentration and profile of these phenolics. Accordingly, viticulturists nowadays seek to optimise growing strategies that favour phenolic biosynthesis.

The growing demand for grapes and their products, such as raisins and juice, has made them directly linked to human life, especially since they occupy the top position among fruit trees in terms of production and cultivated area. The economic importance of grapes is attributed to their significant economic returns, their ability to bear fruit for decades, and their high nutritional value [9]. The fruit contains sugars, vitamins, organic acids, mineral salts, proteins, fats, and other nutrients [10]. Furthermore, grapes are important for medicinal purposes in treating many diseases, as fresh grapes contain the compound resveratrol, which inhibits the growth of cancer cells [11,12].

Foliage foliar fertiliser spray programs Read from memory or augment and enhancement of grape quality, is one potential strategy to improve grapevine attributes by enhancing plant growth rates and yields (increased production) leading to increased crop quality [13]. Foliar nutrition is an important aspect of development in modern agriculture. It has been proved through research and experiments that it is possible to provide plants with different nutrients by spraying solutions of said nutrients, which are subsequently absorbed by leaves and other parts of the plant (above the soil surface) such as stems and fruits [14,15]. Additionally, experimentally, it has been shown that plant extracts and amino acid containing compounds [16] which are an integral component of plant growth and development. Algae (brown, red and green) are a good source of some fertilizer elements in a dry or extract form owing to the high concentration of growth stimulating substances, amino acids and some macroelements as well as vitamins [2].

Agrimax® is a commercial formulation, derived from seaweed extracts have been further incorporated in grape production systems that seek to increase yield and fruit quality while decreasing the use of synthetic fertilizers. It has been reported that these products exert a positive effect on physiological activities such as photosynthesis, nutrient assimilation, and enzyme activity in the phenolic biosynthesis pathway.

Thus, the objective of this work is to study the impact of foliar application of a seaweed extract (Agrimax®) on the accumulation of main phenolic compounds in grapes (*Vitis vinifera* L.) with particular emphasis on gallic acid, caffeic acids, chlorogenic acids and cinnamic acids in two cultivars (Halwani and Kamali). This study aims to contribute to the development of sustainable grape production systems, by clarifying the way natural biostimulants may influence phytochemical quality of grapes.

2. Materials and methods

2.1. Sampling and experimental design

The experiment was carried out at 2024 year on a private sector in Babil Governorate, to study the effect of plant variety and seaweed extract spraying on phenolic contents of grapes. A factorial experiment based on the complete random design, including two factors; first grape varieties (Halwani and Kamali) as a second factor sprayed with seaweed extract Agrimax® (Table 1), at three concentrations: 0 ML/L, 15 Ml/L and 30 Ml/1 in waterwere used. There were 18 replications of each experimental unit, with three plants per replication (a total of 54 plants used in the experiment). The treatments were randomly allocated to experimental units in a complete random way and each experimental unit, consisting of a set if three plants, had the same chance to receive any treatment. Randomization was carried out before field installation using random allocation for the prevention of selection bias and unbiased treatment effect estimation. Sprays of the seaweed extract (5 broad) were conducted, with the 1st on April 1, 2024 and every 15 days afterwards. This spraying treatment was performed in a small hand sprayer early in the morning for complete wetting of the plants.

For this, a CRD was selected as the vineyard area under experimentation presented relatively homogeneous characteristics in relation to soil properties, topography, irrigation schedule and wine age. With such homogeneous environments, CRD maximizes the freedom in treatment allocation and simplifies the analysis of variance, making it possible to compare treatments efficiently without blocking.

Grapes were harvested when commercially mature, based on uniform berry color and achieving the desired total content of soluble solids for each cultivar. Sampling was conducted during harvesting, randomly selecting the bunches from each plant in an experimental unit. In each replicate (from three plants, an identical number of clusters were removed to obtain a sample that is representative and berries were randomly collected from separate branches within the cluster for analyses.

2.2. Environmental conditions

Experimental vineyard. The experimental vineyard was established in Babil Governorate (central Iraq) at the Middle Euphrates region. The site features flat topography and has been cultivated - with grapevines for a long period, leading to homogenous vine age and management history across the experiments area. The vineyard soil type was silty loam clay depth, and it belonged to general alluvial soils in Babil. The soil was moderately fertile, light and well-drained with a good climate for grape growing.

All the vines were irrigated according to the generally applied surface (flood) irrigation system in the experimental site. Irrigation was provided at 7–10 day intervals into the active growth season (March to September) depending on temperature and evaporative demand. Watering was consistent among all treatments to prevent interferences. The irrigation was scheduled to avoid water stress, especially at flowering, berry setting and fruit development.

The growing season of 2024 had a semi-arid climate featuring warm summers and cool spring temperatures. During the foliar application and fruit development season (April–August), mean daily temperatures varied from about 22 to 28 °C during spring, to about 35 to 42 °C at summer, whereas relative humidity was in the low to moderate range on comparison. The experimental period was characterized by minimal rainfall, which occurred mostly in early spring, so the climatic factor had little influence on vine water status during the major growth and fruit set stages. No abnormal climatic events (ie frost, hailstorm, excessive heat beyond normal regional conditions) were reported during the experiment.

At large scale, soil properties, irrigation regime and weather conditions were typical of the experimental area, indicating that possible differences in phenolic composition could be attributed mainly to grape variety and seaweed extract treatments rather than to environmental variability.

2.3. HPLC analysis

Quantitative analysis was performed using high-performance liquid chromatography (HPLC) equipped with a quaternary pump, autosampler, column oven, and UV/Vis detector. Separation was achieved on a reverse-phase C18 column (250 × 4.6 mm, 5 μm particle size) maintained at 25–30 °C.

The mobile phase consisted of:

Solvent A: Water containing 0.1% (v/v) acetic acid

Solvent B: Methanol (HPLC grade)

Elution was carried out using a gradient mode as follows:

0–5 min, 5% B;

5–20 min, 5–40% B;

Table 1. Components of Agrimax® seaweed extract.

Composition (%)	Nitrogen	Phosphorus	Potassium	Amino acids	Organic matter
Value	4	2	2	30	11

20–25 min, 40–95% B;
25–30 min, 95% B, followed by re-equilibration to initial conditions.

The flow rate was set at 1.0 mL/min, and the injection volume was 20 μL. Detection was performed at wavelength 280 nm.

2.4. Calibration and quantification

The calibration curves were prepared with external standards at seven concentration levels (0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 mg/L). All the levels were tested in triplicate and calibration -graphs were made by means of peak area versus concentration. Linear regression analysis showed good linearity in a range of detection and $R^2 \geq 0.995$ were obtained. The estimated contents of analytes in the samples were between 0.08 and 4.12 mg/g fresh weight.

2.5. Method validation

Validation of the HPLC method Validation of the developed HPLC method was done based on established chromatographic validation process which include determination of accuracy, precision, selectivity, matrix effect as well as LOD and LOQ. Reproducibility and precision were assessed with replicate determinations at different concentrations, while selectivity was proved by the lack of interfering peaks of other potential co-existed components in chromatography as investigated at the retention times for each analyte under study. LOD and LOQ were calculated according to the equations related with S/N ratio, which was based on the general acceptable criteria. Matrix effects evaluation was done to ascertain the reliability of quantification in complex plant matrices, as components of the matrix can negatively affect ionization or detector response of an analyte. These validation criteria have been broadly accepted as crucial for guarantying the robustness and the reliability of chromatographic methods, recently being highlighted in studies that focused on their role within analytical toxicology and pharmaceutical analysis [17]. Additionally, it is well established in the literature that assessment of matrix effects and selectivity in chromatographic analysis of complex matrices is critical [18]. Validation results showed that the new method meets the generally theoretical requirements of analytical validation, and it can be used with confidence for quantification purposes.

2.6. Statistical analysis

Data were Excel 2020 recorded and analyzed with GenStat (14 ed.). A 2-way analysis of variance (ANOVA) followed by Šidák test was carried out to assess the main effects of grape variety and seaweed extract concentration, as well as their interactions on the phenolic compounds. A p value of <0.05 was considered significant and means were compared using the Least Significant Difference (LSD) test.

3. Results

3.1. Effect of variety and seaweed extract on caffeic acid content

As it is observed in Table 2 and Fig. 1, grape variety as well as the application of foliar seaweed extracts had a significant effect on caffeic acid concentration in grape methanolic extracts. There was a significant influence of treatment on caffeic acid content, and across treatments Halwani cultivar had higher value of caffeic acid than Kamali cultivar with means of 16.7 and 14.8 mg/L respectively. Foliar application of seaweed extract resulted in a significant dose-dependent increase for caffeic acid content, with the highest concentration (30 ml/L) showing the highest mean value (16.95 mg/L), when compared to control (0 ml/L) treatment (14.35 mg/L). There was a variety vs seaweed extract concentration interaction. Maximum caffeic acid (17.8 mg/L) content was found in Halwani variety at 30 ml/L seaweed extract treatment and as minimum value of 13.3 mg/L in untreated Kamali plants.

3.2. Effect of variety and seaweed extract on gallic acid content

The results shown in Table 3 and Fig. 2 reveal that the grape variety played a significant role on gallic acid concentration in grape methanolic extracts. The Halwani cultivar exhibited higher mean value of gallic acid content (3.1 mg/L) than those recorded

Table 2. Effect of variety and seaweed extract (0, 15 and 30 ml/L) on the concentration of caffeic acid in grape methanolic extract contents (mg/L).

Variety	Seaweed extract			
	0	15	30	Mean
Halwani	15.4	16.9	17.8	16.7
Kamali	13.3	15.2	16.1	14.8
Mean	14.35	16.05	16.95	
$L.S.D_{0.05}$	Variety	Seaweed	Variety * Seaweed	
	1.9	2.3	4.2	

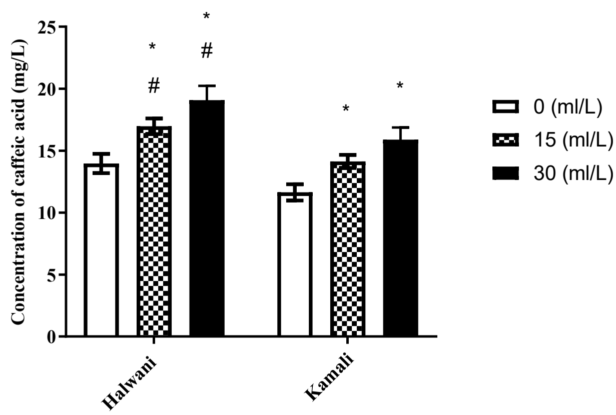

*Fig. 1. Effect of variety and seaweed extract (0, 15 and 30 ml/L) on the concentration of caffeic acid in grape methanolic extract contents (mg/L). Two-way ANOVA with Sidak's multiple comparisons test, *: Significant (p<0.05) compared to control (0 ml/L) in the same group, #: Significant (p<0.05) compared to the corresponding concentration in Kamali, n = 18 ± SEM.*

Table 3. Effect of variety and seaweed extract on the concentration of gallic acid in grape methanolic extract contents (mg/L).

Variety	Seaweed extract			
	0	15	30	Mean
Halwani	2.3	3.1	3.9	3.1
Kamali	1.9	2.5	2.8	2.4
Mean	2.1	2.8	3.3	
$L.S.D_{0.05}$	Variety	Seaweed	Variety * Seaweed	
	0.3	0.5	0.9	

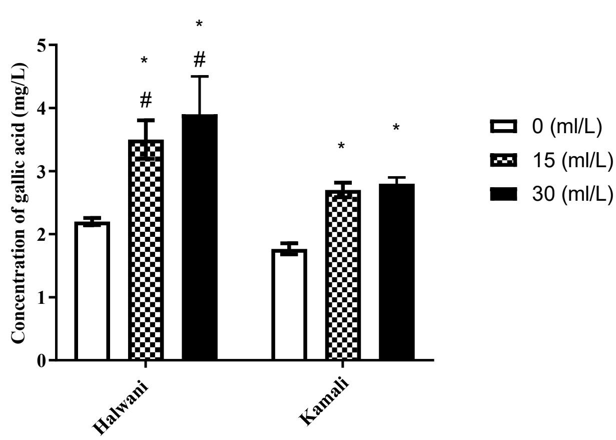

*Fig. 2. Effect of variety and seaweed extract (0, 15 and 30 ml/L) on the concentration of gallic acid in grape methanolic extract contents (mg/L). Two-way ANOVA with Sidak's multiple comparisons test, *: Significant (p<0.05) compared to control (0 ml/L) in the same group, #: Significant (p<0.05) compared to the corresponding concentration in Kamali, n = 18 ± SEM.*

by the Kamali cultivar (2.4 mg/L). Moreover, foliar application of seaweed extract increased gallic acid content in a dose-dependent manner whose rise was linear with the increase in the extract concentration. The highest mean content of gallic acid (3.3 mg/L) was found at 30 ml/L, compared to the control plants which had the lowest content (2.1 mg/L. The grape variety and seaweed extract concentration interaction was significant and Halwani variety sprayed with 30 ml/L gave the highest gallic acid content (3.9 mg/L). In untreated plants, the lowest concentration of gallic acid was recorded in Kamali (1.9 mg/L).

3.3. Effect of variety and seaweed extract on cinnamic acid content

Results presented in Table 4 and Fig. 3 show that grape variety and seaweed extract concentration also had a significant impact on cinnamic acid content of grape methanolic extracts. Between the two varieties, Halwani had significantly higher cinnamic acid at average values of 1.4 and 0.9 mg/L respectively. The cinnamic acid content was significantly higher in leaves with foliar applications, for the 30 ml/L dose being the most efficient (highest mean value = 1.9 mg/L), when compared to control (0.6 mg/L). There was a significant variety and seaweed extract application interaction effect. The maximum cinnamic acid concentration (2.4 mg/L) was obtained in Halwani plants treated with 30 ml/L of seaweed extract, while the minimum (0.5 mg/L) was found in untreated Kamali plants.

3.4. Effect of variety and seaweed extract on chlorogenic acid content

In the case of grape methanolic extracts (Table 5, Fig. 4), no influence on chlorogenic acid concentration was observed only because of grape variety. Yet, seaweed extract was effective in the elevation of chlorogenic acid content in both varieties when foliar applied. At 30 ml/L, the maximum average - chlorogenic acid content (3.45 mg/L) was observed, and it was highest in plants treated with biostimulant but not saline compared to the control (1.8 mg/L). In spite of the absence of a significant main effect for variety, a significant interaction between grape variety and seaweed extract concentration was observed. Halwani type treated with 30 ml/L seaweed got the maximum chlorogenic acid at 30 ml/L (3.8 mg/L) and the minimum was observed in untreated Kamali variety (1.7 mg/L).

3.5. Effect of variety and seaweed extract on p-coumaric acid content

Two-way ANOVA (Fig. 5) demonstrated a significant effect of the experimental factors on p-

Table 4. Effect of variety and seaweed extract in the concentration of cinnamic acid in grape methanolic extract contents (mg/L).

Variety	Seaweed extract			
	0	15	30	Mean
Halwani	0.7	1.2	2.4	1.4
Kamali	0.5	0.8	1.5	0.9
Mean	0.6	1.0	1.9	
L.S.D$_{0.05}$	Variety	Seaweed		
	0.2	0.5	Variety * Seaweed 1.2	

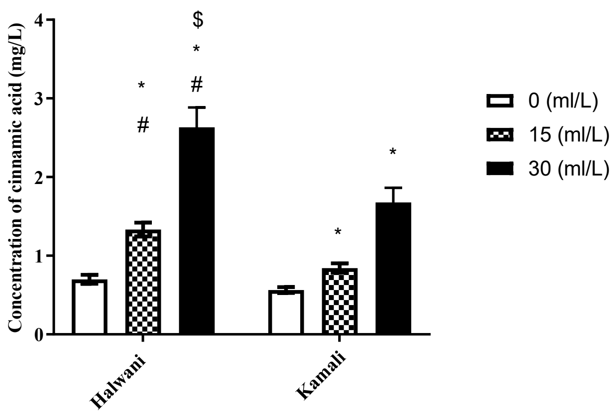

*Fig. 3. Effect of variety and seaweed extract (0, 15 and 30 ml/L) on the concentration of cinnamic acid in grape methanolic extract contents (mg/L). Two-way ANOVA with Sidak's multiple comparisons test, *: Significant (p<0.05) compared to control (0 ml/L) in the same group, $: Significant (p<0.05) compared to (15 ml/L) in the same group, #: Significant (p<0.05) compared to the corresponding concentration in Kamali, n = 18 ± SEM.*

Table 5. Effect of variety and seaweed extract on the concentration of chlorogenic acid in grape methanolic extract contents (mg/L).

Variety	Seaweed extract			
	0	15	30	Mean
Halwani	1.9	2.7	3.8	2.8
Kamali	1.7	2.3	3.1	2.3
Mean	1.8	2.5	3.45	
L.S.D$_{0.05}$	Variety	Seaweed	Variety * Seaweed	
	N.S	1.5	2.9	

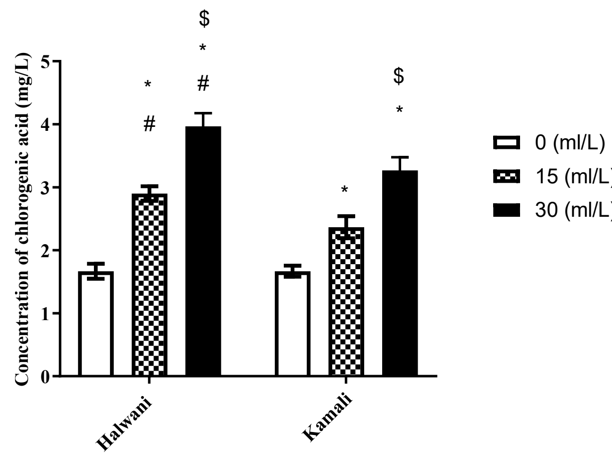

*Fig. 4. Effect of variety and seaweed extract (0, 15 and 30 ml/L) on the concentration of chlorogenic acid in grape methanolic extract contents (mg/L). Two-way ANOVA with Sidak's multiple comparisons test, *: Significant (p<0.05) compared to control (0 ml/L) in the same group, $: Significant (p<0.05) compared to (15 ml/L) in the same group, #: Significant (p<0.05) compared to the corresponding concentration in Kamali, n = 18 ± SEM.*

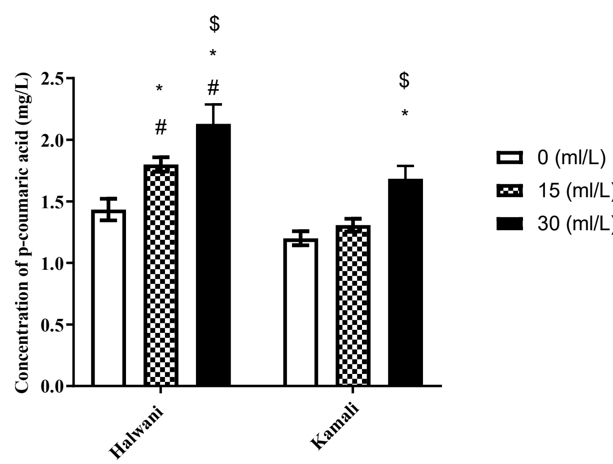

*Fig. 5. Effect of variety and seaweed extract (0, 15 and 30 ml/L) on the concentration of p-coumaric acid in grape methanolic extract contents (mg/L). Two-way ANOVA with Sidak's multiple comparisons test, *: Significant (p<0.05) compared to control (0 ml/L) in the same group, $: Significant (p<0.05) compared to (15 ml/L) in the same group, #: Significant (p<0.05) compared to the corresponding concentration in Kamali, n = 18 ± SEM.*

coumaric acid concentration ($p < 0.05$). However, post hoc comparison by analyzing LSD test (Table 6) proved that no significant difference was noted between treatment means, suggesting that the level of variation was not large enough to easily distinguish the individual treatments.

4. Discussion

The results indicated that both grape variety and seaweed extract foliar spray have strong influence on the phenolic acid composition of grape methanolic extract. Phenolics including caffeic, gallic and cinnamic, chlorogenic and p-coumaric acids contribute significantly in grape nutritional richness, antioxidant capacity and possible nutraceutical properties. Their levels are also largely determined by genetic, environmental, and agronomic conditions [19,20]. The difference between the two grape varieties in the content of some phenolic compounds in their fruits is often due to

Table 6. Effect of variety and seaweed extract on the concentration of p-coumaric acid in the grape methanolic extract contents (mg/L).

Variety	Seaweed extract			
	0	15	30	Mean
Halwani	1.4	1.8	2.1	1.7
Kamali	1.1	1.3	1.6	1.3
Mean	1.25	1.55	1.85	
$L.S.D_{0.05}$	Variety	Seaweed	Variety * Seaweed	
	N.S	N.S	N.S	

the genetic ability of the variety to express itself in its growing environment, such as varying weather conditions, soil content, and quality. These results are consistent with previous studies [21,22].

In all phenolic acids tested—caffeic, gallic, cinnamic and (less) chlorogenic acid—the Halwani variety was consistently characterized by higher levels of these compounds than the Kamali one. This is consistent with previous reports indicating that genotype has a major influence on the control of phenolic biosynthesis, due to the differences in metabolic capacity, enzyme activity and phenylpropanoid pathway in varieties [23,24]. The higher phenolic content of Halwani might be due to better genetic potential in activating for phenylalanine ammonia-lyase (PAL) and resistance against environmental stress conditions, factors that stimulate the production of phenolic compounds [25].

The consistent rise of caffeic, gallic, cinnamic and chlorogenic acids after foliar application with seaweed extract suggests that the physiological response to exposure to bioactive compounds in seaweed extract are positive. Seaweed extracts have macro- and microelements, amino acids, polysaccharides, and plant growth hormones such as cytokinins and auxins [26]. These elements promote plant metabolism increase nutrient absorption and activate enzymatic reactions in phenolic synthesis. Nitrogen, in particular, is important in the promotion of RNA and protein synthesis which triggers growth and division of meristematic tissues as well as being a precursor for amino acids such as tryptophan that is the substrate for auxin-formation [27]. These physiological activities may together result in the improvement of phenolic metabolism within grape berries [28].

The dramatic increases in phenolic acids particularly at 30 ml/L concur with previous reports demonstrating positive seaweed extracts can increase the amount of phenolics, antioxidant activity and fruit quality in grapes and other fruits [25,29]. This trend was further reinforced by the variety foliar interaction effect, with the Halwani variety showing significantly higher increases in most phenolic compounds. This finding indicates that some cultivars might have more sensitivity to biostimulants depending on leaf shape, stomatal features or an amalgamation of metabolic capacity.

In comparison, no significant differences were observed for p-coumaric acid among treatments. This perhaps suggests that p-coumaric acid may be more genetically controlled, nutritional insensitive or exists in stable concentrations of the phenylpropanoid pathway. Consistent stability of p-coumaric acid levels have also been reported in other grape studies indicating commonality of the metabolite function [30].

In addition to amino acid provision, seaweed extracts contain naturally occurring plant growth regulators, notably cytokinins, auxins, and gibberellin-like compounds [31]. Cytokinins enhance photosynthetic capacity by delaying leaf senescence, increasing chlorophyll stability, and improving carbon assimilation, thereby increasing the pool of photosynthates available for secondary metabolite production [32]. Auxins, derived from tryptophan metabolism, influence cell expansion and vascular differentiation and indirectly stimulate phenolic accumulation by activating stress-responsive and developmental signaling networks linked to phenylpropanoid metabolism [33]. These hormonal signals have been shown to upregulate genes encoding PAL, cinnamate-4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL), which collectively regulate the downstream formation of hydroxycinnamic acids.

In addition, seaweed extracts serve as biostimulants that elicit a mild physiologic stress or "elicitor-like" responses to trigger plant defense systems [34]. Phenolic compounds are among the plant's defense mechanisms and often accumulate following increases in antioxidant requirements. The seaweed-extracted polysaccharides and oligosaccharides may also act as signaling molecules, and induce reactive oxygen species (ROS)-dependent signaling cascades for activation of phenolic biosynthesis in response to oxidative stress.

The dose-response increase in phenolic acids was more apparent, especially at 30 ml/L, and higher concentrate of seaweeds extract possibly induced the synergetic effects of nutrient supply, hormone regulation and metabolic response. The higher response to the treatment in Halwani suggests a genotypic-specific sensitivity of biostimulant application, thus likely differentiating enzyme activity or transcriptional regulation of phenyl-propanoid genes and general metabolic potential. Together, these pathways combine to form a reasonable bio-chemical explanation for the increased accumulation of phenolic compounds in grape berries after foliar application of seaweed extract.

In contrast to other phenolic acids studied in this work, the content of p-coumaric acid was unaffected by grape variety, seaweed extract concentration or their interaction. This stability could be due to the central metabolite status of & p-coumaric acid in the phenylpropanoid pathway and its high turnover. p-Coumaric acid is a central intermediate, and not just the end storage metabolite, that gives rise to other subsequent compounds for several metabolic pathways including caffeic acid, ferulic acid, chlorogenic acids, flavonoids, lignin-related polymers [35]. Consequently, enhanced pathway flux can drive the rapid conversion of p-coumaric acid to downstream products rather than its accumulation as the free form.

In addition, the biosynthesis of p-coumaric acid is finely modulated by constitutive activity of enzymes such as cinnamate-4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL), that quickly redirect this intermediate toward downstream products which are required for constructional or defensive use [36]. Therefore, if phenylpropanoid activity is being upregulated by seaweed extract it may selectively direct more flux towards downstream phenolics such as caffeic and chlorogenic acids (as observed in the present work) leaving unchanged levels of p-coumaric acid. Furthermore, p-coumaric acid is frequently present primarily in bound or esterified form in plant cell walls and conjugated complexes, rather than as a free soluble compound. These bound pools are less responsive to rapid agronomic treatments, such as foliar application of the biostimulant, and their presence might not be totally revealed in methanolic extracts directed towards free phenolics [37]. This biochemical feature may also account for the absence of measurable variation across treatments.

Taken together, the lack of significant response of p-coumaric acid suggests that its concentration is under stronger metabolic and structural control than that of other phenolic acids, and that seaweed extract application primarily enhances the accumulation of downstream phenolic metabolites rather than early pathway intermediates.

5. Conclusions

It can be concluded that the variety can influence the composition of grapes with high phenolic contents observed in Halwani. In addition, our results reported that seaweed extract can influence the contents of grapes. Furthermore, the combined effect of the two study factors further improved the grape content of some phenolic compounds. Future studies would include expanding the range of biostimulants such as humic acids, amino acid formulations to compare their efficiency with Agrimax extract. Also, evaluation of a broader set of grape varieties and additional phenolic and antioxidant compounds may be performed.

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This work receives no funding.

Conflict of Interest

Authors declare no conflict of interest.

Ethical Approval

The study exclusively involved plant material and did not include human or animal subjects, formal ethical approval was not required. The ethics statement has therefore been removed from the manuscript.

Data Availability

No other dataset to declare.

Author contributions

All authors contributed equally to the manuscript including data collection, analysis and writing.

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