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Pyroligneous Acid Affects Grapevine Growth, Yield, and Chemical Composition of Leaf, Pomace, and Juice

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ABSTRACT: In the past decade, many studies have investigated the effects of biostimulants on viticulture. However, the impact of pyroligneous acid (PA) on grape (*Vitis vinifera*) production has not yet been reported. In this study, PA at varying concentrations (0, 4, 8, and 12% PA) and application frequencies (14-, 21-, and 28-day intervals) were applied to enhance the growth, yield, and quality of grapes (cv. KWAD7-1). The results showed that the treated grapes responded differently to PA application. The 4 and 8% PA showed a nonsignificant ($p > 0.05$) increase in yield of about 0.37- and 0.18-fold, respectively, compared to the 0% PA. The 12% PA, on the other hand, reduced the yield by approximately 0.03-fold compared to the 0% PA. Carotenoid, total phenolics, flavonoid, and sugar were altered by the PA. Interestingly, the 4% PA significantly ($p < 0.05$) improved total carotenoids (0.34-fold), total phenolics (0.26-fold), and flavonoids (0.26-fold) compared to the 0% PA. The 4% PA applied at 21-day and 28-day intervals remarkably improved vine and leaf growth, respectively. In conclusion, the 21-day interval of PA application significantly ($p < 0.05$) improved fruit fresh weight, juice weight, juice volume, press weight, °Brix, pH, salinity, total dissolved solids, electrical conductivity, and titratable acidity. Further study is necessary to assess how PA can influence the metabolites present in grape wine.

KEYWORDS: *Vitis vinifera, pyroligneous acid, biostimulant, optimal application, productivity, metabolite accumulation*

INTRODUCTION

Sustained global climate change and shift in growing season have left millions vulnerable to severe food insecurity.[1] To achieve global food and nutritional security, it is essential to increase the productivity and quality of crops, especially fruits and vegetables.[2] The horticultural sector has been tasked with improving crop production to meet the increasing food demand caused by the global population's growth, which is anticipated to reach 9.7 billion by 2050.[3] Crop output is expected to increase by approximately 50−70% to meet this ever-increasing food demand.[4,5] In response, farmers have increasingly relied on agrochemicals, including fertilizers and pesticides, leading to many human health issues, contamination of surface and groundwater, environmental degradation, and increased costs of production.[5−7] According to ref 8, the horticultural sector has emerged as a leading contributor to nitrogen and phosphorus pollution affecting terrestrial, freshwater, and marine ecosystems. Fruit production, for instance, requires repeated application of synthetic fungicides throughout the growing period.[9] Crop production contributes nearly 13% to global greenhouse gas emissions, playing a remarkable role in driving global warming and climate change.[6] To safeguard and improve the natural resource base while boosting productivity, new and creative systems are essential.[6,10] In the last 3 decades, a range of advanced techniques and tools have been developed to improve the sustainability of agricultural production systems by remarkedly reducing the use of synthetic agrochemicals like pesticides and fertilizers.[11] One innovative approach involves utilizing pyroligneous acid (PA), a natural and eco-friendly byproduct derived from the pyrolysis of plant biomass, as an alternative or supplement to synthetic agrochemicals.[12−16] Thus, it can promote plant growth and development, and improve soil health with a lower toxic residue in the environment compared to many synthetic agricultural inputs[14,17]

Pyroligneous acid has long been utilized in agriculture for its roles as a biostimulant, biofertilizer, biopesticide, and antimicrobial agent.[18,19] Studies have shown that PA improves seed germination, plant growth, and crop yield by enhancing fruit size or diameter, fruit weight, and quality of several crops and vegetables due to butanolide present in it as bioactive compound.[14,19−21] For instance, PA at 30% improved soybean (*Glycine max*) yield,[22] applying a 20% PA solution as a drench improved the performance of rockmelon (*Cucumis melo*)[23] and its foliar application improved growth and yield of lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), and cole (*Brassica oleracea*).[24] It could be deduced from the various studies that PA effectiveness depends on the mode of application such as concentration, the frequency of application, and plant species.[14,25] Concentrations of PA used in current studies vary from 0.01 to 30% and frequencies

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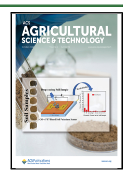

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Figure 1. Location of the experimental vineyard from Google Earth. The vineyard boundaries are shown with four yellow points.

of application can be 7-, 14-, 21-, or 28-day intervals.[14,22,23,25,26] The wide range of PA concentrations used in previous studies depends on the mode of application, thus either by drenching or foliar application and the type of crops. However, studies on the biostimulatory effects of PA on grapes have not been reported yet.

Grape berries (*Vitis vinifera*) are nonclimacteric fleshy fruits with great economic value.[27,28] They are one of the most cultivated horticultural crops worldwide and used as food and in wine production.[29] According to ref 30, about 71% of grapes are utilized for wine production, while 27% are consumed as fresh fruit, and 2% are processed into raisins or dried fruits. In addition, grape seeds and grape pomace have been utilized in the food industry as functional food, and in food processing.[31,32] They are also used as cosmetics, pharmaceuticals, and supplements.[32] The chemical composition of grape berries is complex and contains hundreds of compounds and made up of 75−80% water, organic acids, amino acids, sugar, phenolic compounds, and proteins.[33] The nutritional and health properties of grapes have been linked to the prevention of various chronic diseases, including obesity, diabetes, cancer, and cardiovascular conditions,[31,34,35] due to grape chemical composition. However, grape production is constrained by biotic and abiotic stresses, such as drought, soil infertility, insect pests, and diseases.[36,37] PA can be explored as a sustainable strategy to manage these challenges in order to promote grape production. Thus, PA can help control pests and diseases and improve the uptake of nutrients which can aid the grapevines to better withstand various stresses and also improve productivity. It is therefore important to note that further research is needed to optimize its application in grape production. The aim of this study was to identify the optimal concentration and application frequency of PA to enhance grape plant growth, yield, juice quality, and chemical composition.

MATERIALS AND METHODS

Field Location. A field experiment was carried out in Jost Vineyard, Malagash (latitude 45°46′N; longitude 63°25′W; altitude 45 m above sea level), Nova Scotia, Canada (Figure 1).

The studies were performed between May and August 2022 and 2023 growing seasons. The mean annual precipitation and temperature during the studies ranged from 4.5 to 9.8 mm and 9.5 to 20 °C, respectively.[38]

Experimental Design, Treatment Application, and Field Management. An established vineyard of approximately 23-year-old grape (*V. vinifera* cv. KWAD7-1) plants with 1.2 m between plants and 3 m between plant rows was utilized in the experiment. The experimental treatments were arranged in a randomized complete block design (RCBD) with four replications and five plants for each treatment per replication ($N = 20$). Proton Power, Inc. (Lenoir City) provided the PA used in this experiment, and it was made from the biomass of white pine (*Pinus strobus*) using a fast pyrolysis process.[14] Dilutions of the PA and water were prepared by using the raw PA (100%) and water to obtain the various concentrations on a volume per volume (v/v) basis. The PA concentrations were 0% (i.e., only water as control) and 4, 8, and 12% applied to the leaves of the plants at a 21-day interval in the first year. In the second year, only 4% of PA was applied at 14-, 21-, and 28-day intervals. The treatment application commenced at the buds break stage, and each plant was sprayed with 0.1 L of PA solution. At the vegetative stage and reproductive stages, approximately 0.3 and 0.4 L of each treatment were applied per plant. The plants were rainfed throughout the study. Weed control and pruning were done when necessary.

Morpho-Physiological Response. Vine and leaf elongations were assessed by measuring the lengths of four tagged vines and leaves per plant (two plants per replication) weekly after the first treatment application using a ruler. LI-3100 leaf area meter (LI-COR, Inc. Lincoln, Nebraska) was used to measure leaf area. A SPAD 502-plus chlorophyll meter (Spectrum Technologies, Inc.) was used to assess the chlorophyll content on the same tagged leaves. Chlorophyll fluorescence parameters, including maximum quantum efficiency (F_v/F_m) and potential photosynthetic capacity (F_v/F_o), were evaluated on the same leaves using a Chlorophyll fluorometer (Optical Science).

Yield and Juice Quality. The grape clusters were hand-harvested from three middle plants of each replication at maturity approximately 16 weeks after PA application when the berries were soft to touch and the average °Brix was between 13 and 16. Randomly selected samples, each containing 500 berries, were gathered from each experimental plot. Berries that were free from disease and bruising were randomly collected from the upper, middle, and lower sections of the selected clusters. An Ohaus navigator portable balance (ITM Instruments, Inc., Canada) was used to measure the fresh weight of the berries. The sampled berries were immediately transported to the laboratory for juice extraction and quality assessment. The juice was extracted by using a small-scale fruit press, a method used in ref 39 with slight modification. The juice quantity was measured with a measuring cylinder, and the juice weight and press weight were assessed using an Ohaus navigator portable balance (ITM Instruments, Inc., Canada). A handheld refractometer (Atago, Japan) was used to measure the °Brix content

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of the harvested berries. The pH, salinity, total dissolved solids (TDS), and electrical conductivity (EC) of the juice were measured using the ExStik II multipurpose pH meter (model EC 500) from EXTECH Instruments, based in Taiwan. All measurements were performed in three replicates in 50 mL beakers[14] with slight modification. To determine the titratable acidity (TA), 5 mL samples of grape juice from each treatment were diluted in 100 mL of distilled water. The titratable acidity was then measured by titrating the diluted juice with 1 M sodium hydroxide (NaOH) until the pH reached 8.1. The mean titratable acidity was calculated and expressed as a percentage of tartaric acid.[40] Samples from the treated grapes including the pomace and leaf were immediately frozen in liquid nitrogen, ground into powder, and stored in a −80 °C freezer together with the juice until analyzed for biochemical composition.

Estimation of Total Carotenoids. Total carotenoids in the grape juice, pomace, and leaf were determined as described in ref 41. 1 mL of the juice and 0.2 g of ground samples were placed into a sterile 2 mL Eppendorf tube, followed by the addition of 1 and 1.5 mL of 80% acetone, respectively. The mixtures were vortexed for 1 min and then centrifuged at 12,000*g* for 15 min. Subsequently, 200 μL of the supernatant was transferred to a microplate, and the absorbance (A) was recorded at wavelengths of 646.8 and 663.2 nm using a UV−vis spectrophotometer (Jenway, Staffordshire, U.K.), with 80% acetone serving as the blank. For carotenoid analysis, absorbance was measured at 470 nm and calculated by using the appropriate formula:

$$\text{carotenoid } (\mu\text{g/mL}) = \frac{(1000 \times A_{470} - 1.8 \times \text{chl a} - 85.02 \times \text{chl b})}{198}$$

The carotenoid concentration was reported in micrograms per gram of fresh weight (μg/g of FW).

Estimation of Total Phenolics. Total phenolic content (TPC) was assessed using the Folin-Ciocalteu method as described in ref 42 with slight modification. Initially, 1 mL of juice and 0.2 g of ground samples for each treatment were mixed with 1 and 1.5 mL of ice-cold 95% methanol, respectively, and left to incubate in the dark at room temperature for 48 h. Following this, the mixture was centrifuged at 15,000*g* for 15 min, and 100 μL of the supernatant was placed into a new Eppendorf tube. To this, 200 μL of 10% Folin-Ciocalteu reagent was added, and the solution was vortexed for 5 min. Subsequently, 800 μL of 700 mM sodium carbonate was incorporated, followed by another vortexing for 1 min, and the mixture was allowed to stand at room temperature for 2 h. The absorbance of the final solution was measured at 765 nm. TPC was calculated using a standard curve based on gallic acid equivalents, and results were expressed as milligrams of gallic acid equivalents per gram of the sample.

Estimation of Total Flavonoids. Total flavonoids were quantified using a modified method based on previous study in ref 43. A 1 mL aliquot of juice and 0.2 g of ground samples were mixed with 1 and 1.5 mL of 95% methanol, respectively. The mixtures were then centrifuged at 15,000*g* for 15 min, and 500 μL of the resulting supernatant was placed into new tubes. Each tube received 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixtures were vortexed and incubated at room temperature for 30 min, after which the absorbance was measured at 415 nm against a blank. The total flavonoid content was determined using a quercetin standard curve calculated via the appropriate formula:

$$\text{flavonoids } (\mu\text{g/g}) = \frac{C_{\text{flavonoids}} \times V_{\text{extract}}}{m_{\text{extract}}}$$

where $C_{\text{flavonoids}}$ is the concentration of flavonoids (μg/mL), V_{extract} is the total volume of methanolic extract (mL), and m_{extract} is the mass of extract (g).

Estimation of Total Sugar. The phenol−sulfuric acid method described in ref 44 was used to quantify the total sugar in the grape samples. In brief, 1 mL of the juice and 2 g of the ground sample were mixed with 1 and 1.5 mL of 90% ethanol, respectively, to extract the sugars. The mixtures were incubated in a water bath at 60 °C for 60 min to facilitate the extraction process. The volume of the mixture was adjusted to 10 mL with 90% ethanol and centrifuged at 12,000*g* for 3 min to separate the insoluble components. 1 mL of the supernatant was thoroughly mixed with 1 mL of 5% phenol solution in a thick-walled glass test tube. 5 mL of concentrated sulfuric acid was added to the mixture, which was then vortexed for 20 s to ensure proper mixing. The reaction mixture was incubated in the dark for 15 min to allow the color development. The samples were cooled to room temperature (approximately 22 °C) before the absorbance was measured at 490 nm using a UV−vis spectrophotometer. A sugar standard curve, typically using glucose as the reference, was used to quantify the total sugar content, which was expressed as micrograms of glucose per gram of fresh weight.

Statistical Analysis. The data collected underwent analysis of variance (ANOVA) utilizing Minitab version 21 (Minitab, Inc., State College, PA). The means were differentiated through the Fisher least significant difference (LSD) test at a significance level of $p < 0.05$. Additionally, bivariate correlation analysis and two-dimensional (2D) principal component analysis (PCA) were conducted using XLSTAT version 2022.3 (Addinsoft, New York). Graphical representations were created using Microsoft Excel.

RESULTS

Morpho-Physiological Response. The findings of the current investigation revealed that applying PA at varying frequencies remarkably influenced grapevine and leaf elongations (Figure 2).

The application of PA at varying frequencies led to significant ($p < 0.05$) changes in the grapevines. The vine elongations for 14- and 28-day intervals increased rapidly from week 1 to 3, and then for the rest of the weeks, a slow

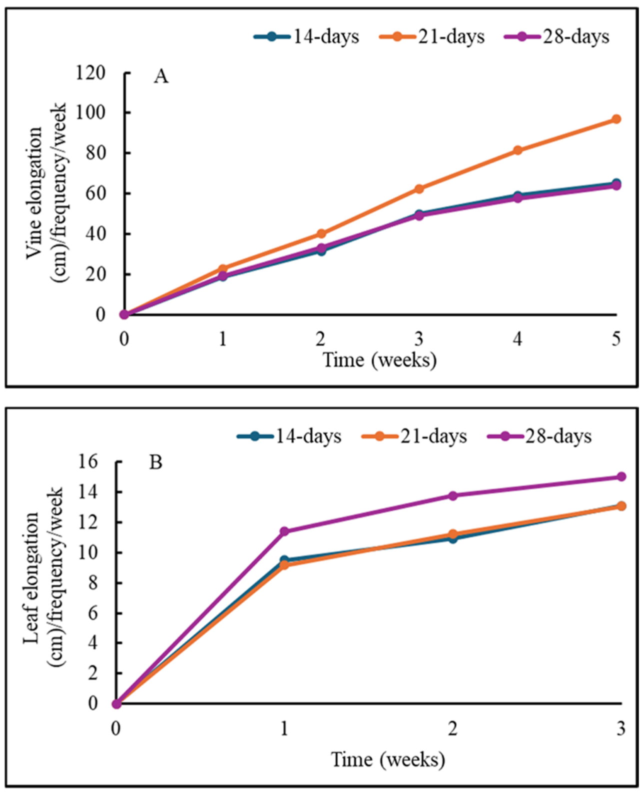

Figure 2. Grapevine response to varying frequencies of PA application. Weekly assessment of (A) vine elongation and (B) leaf elongation following PA application.

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Table 1. Morpho-Physiological Response of Grape Plants Treated with PA[a]

frequencies (days)	vine length	leaf length	leaf area	SPAD	F_v/F_m	F_v/F_o
14	90.33[a]	20.92[a]	255.17[a]	32.35[a]	0.77[a]	3.49[a]
21	122.33[a]	21.41[a]	201.67[a]	31.21[a]	0.74[a]	2.97[a]
28	88.61[a]	22.58[a]	228.43[a]	32.02[a]	0.75[a]	3.11[a]
p-value	0.109	0.300	0.148	0.474	0.331	0.358

[a]Means that do not share an alphabetical letter are significantly ($p < 0.05$) different according to Fisher least significant difference (LSD) at α = 0.05 level of significance.

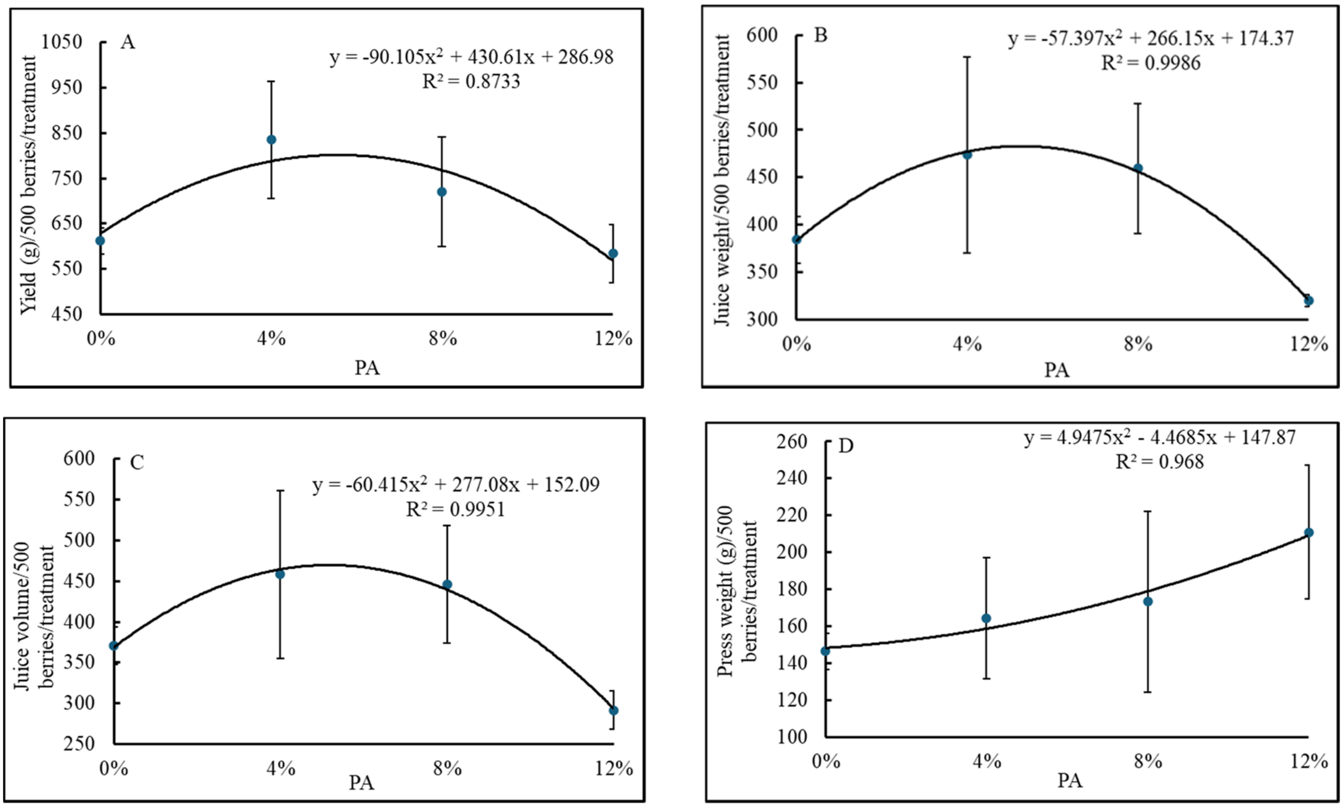

Figure 3. Response of grapevine plant to varying rates of PA application. Changes in (A) yield, (B) juice weight, (C) juice volume, and (D) press weight following 0, 4, 8, and 12% PA application rates. Error bars represent the standard deviation.

increment in length was observed. The grapevines treated at 21-day intervals significantly ($p < 0.05$) increased length throughout the study, i.e., from week 1 up to week 5 (Figure 2A). In addition, leaf elongation of the treated grapes was increased with the variation in the application time of the PA. Grape plants treated at 14- and 21-day intervals had the lowest leaf elongation. The 14- and 21-day intervals had a rapid increment in leaf length in week 1 and continued slowly for the rest of the weeks. Interestingly, unlike the vine elongation, the application of PA at 28-day interval significantly ($p < 0.05$) increased leaf elongation for 3 weeks of observation. Similar to the 14- and 21-day intervals, the 28-day interval had a rapid increment in leaf length in week 1 and continued slowly for the rest of the weeks (Figure 2B).

At maturity, i.e., 14 weeks after treatments application, the treatments applied at different times showed no significant ($p > 0.05$) effects on vine length, leaf length, leaf area, chlorophyll content (SPAD), maximum quantum efficiency (F_v/F_m), and potential photosynthetic capacity (F_v/F_o) (Table 1).

Vine length was nonsignificantly ($p > 0.05$) increased slightly with PA applied at 21-day interval compared to 14- and 28-day intervals. The highest leaf length was recorded with PA applied at a 21-day interval, followed by 21- and 14-day intervals. The leaf area was nonsignificantly ($p > 0.05$) higher with PA applied at 14-day interval, followed by 28- and 21-day intervals. The 14-day interval of PA application had a nonsignificant ($p > 0.05$) highest chlorophyll content, followed by 28- and 21-day intervals. The application of PA at 14-day interval recorded nonsignificantly ($p > 0.05$) the highest maximum quantum efficiency (F_v/F_m) and potential photosynthetic capacity (F_v/F_o) compared to the 28- and 21-day intervals for PA application (Table 1).

Grape Berry Yield and Quality. The grape plants responded differently to the different concentrations and application frequencies of PA. It was observed that berry yield, juice weight, juice volume, and press weight assessed were not different ($p > 0.05$) for all of the PA treatments. However, the 4 and 8% PA showed a remarkable increase in yield of about 0.37- and 0.18-fold, respectively, compared to the 0% PA. The 12% PA, on the other hand, reduced berry yield by approximately 0.03-fold compared to the 0% PA (Figure 3A).

The 4 and 8% PA treatments significantly ($p < 0.05$) increased juice weight by approximately 0.23- and 0.20-fold,

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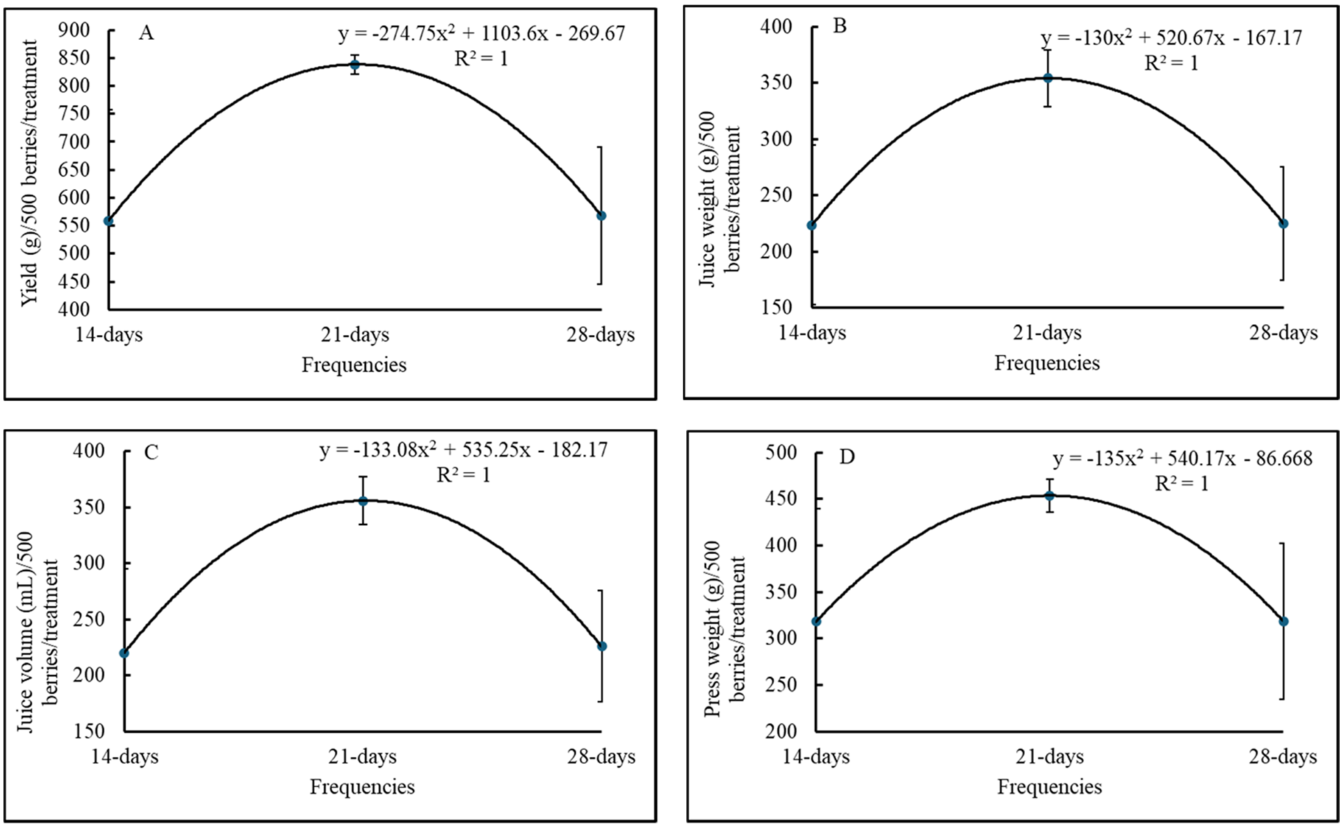

Figure 4. Grapevine response to varying frequencies of PA application. Change in (A) yield, (B) juice weight, (C) juice volume, and (D) press weight following PA application at 14-, 21-, and 28-day intervals. Error bars represent standard deviation.

Table 2. Juice Quality Indices Response[a]

frequencies (days)	°Brix	pH	salinity (mg/L)	TDS (mg/L)	EC (mS/cm)	TA (%)
14	12.60[c]	3.38[c]	936.00[c]	1311.33[c]	1.89[b]	0.35[b]
21	13.10[a]	4.31[b]	1190.00[a]	1700.00[a]	2.45[a]	0.45[a]
28	12.80[b]	4.53[a]	1043.33[b]	1553.33[b]	2.42[a]	0.4[ab]
p-value	*	0.000	0.000	0.000	0.049	0.016

[a]Means that do not share an alphabetical letter are significantly ($p < 0.05$) different according to Fisher least significant difference (LSD) at α = 0.05 level of significance. * indicates a *p*-value less than or equal to 0.05, suggesting statistical significance.

respectively, while the 12% PA reduced it by about 0.17-fold compared to the 0% PA (Figure 3B). In addition, the 4 and 8% PA showed increase in juice volume by approximately 0.24- and 0.20-fold, respectively, and the 12% PA decreased the juice volume by about 0.21-fold compared to the 0% PA (Figure 3C). Interestingly, all of the PA treatments (i.e., 4, 8, and 12%) increased the press weight by about 0.12-, 0.18-, and 0.44-fold compared to the 0% PA treatment (Figure 3D).

The 21-day interval of PA application significantly ($p < 0.05$) improved fruit fresh weight, juice weight, juice volume, and press weight (Figure 4A–D).

The 21-day intervals of PA application significantly ($p < 0.05$) improved °Brix, pH, salinity, total dissolved solids (TDS), electrical conductivity (EC), and titratable acidity (TA) (Table 2).

Grape Leaf, Pomace, and Juice Biochemicals. The results from the chemical analysis of the treated grape plants showed that PA influenced the chemical compounds of the grape leaves, pomace, and juice. The 4% PA showed the same total carotenoid level in the grape juice compared to the 0% PA statistically. However, the 4% PA improved the carotenoids in the juice by approximately, 0.34-fold compared to the 0% PA (Figure 5A).

The PA at 8% also had the same carotenoids as the 0% PA statistically. However, the 8% PA reduced the total carotenoids of the juice by 0.32-fold compared to the 0% PA. The 12% PA significantly ($p < 0.05$) reduced the carotenoids in juice by approximately 0.80-fold compared to the 0% PA (Figure 3A). The total phenolics of the grape juice was significantly ($p < 0.05$) increased by approximately 0.26-, 0.15-, and 0.13-fold following the application of 4, 8, and 12% PA respectively (Figure 5B). Similarly, total flavonoid of the grape juice was significantly ($p < 0.05$) increased by approximately 0.26-, 0.15-, and 0.15-folds following the application of 4, 8, and 12% PA, respectively (Figure 5C). The sugar content of the grape juice was statistically ($p > 0.05$) the same for 0, 4, and 8% PA. However, the 4 and 8% PA reduced the sugar content by about 0.01- and 0.03-fold, respectively, compared to the 0% PA. Conversely, the 12% PA significantly ($p < 0.05$) reduced the sugar in the juice by approximately 0.32-fold compared to the 0% PA (Figure 5D).

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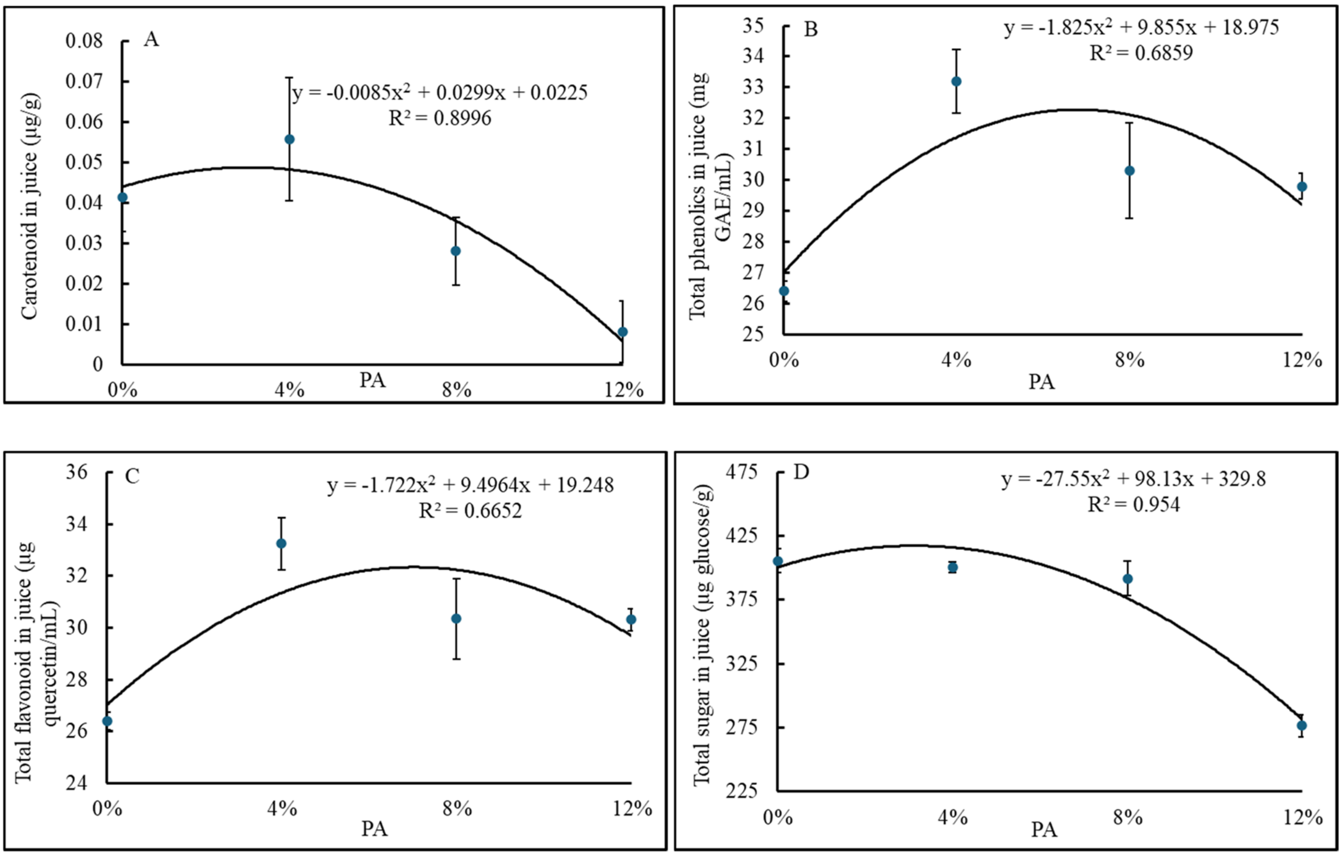

Figure 5. Grapevine response to varying frequencies of PA application. Change in (A) carotenoid, (B) total phenolics, (C) total flavonoid, and (D) total sugar following 0, 4, 8, and 12% PA application rates. Error bars represent standard deviation.

Chemical Composition of the Grape Plant Leaves. Carotenoids determined in the grape plant leaves were significantly ($p < 0.05$) different for the three PA application frequencies (Figure 6A).

The 21-day interval had the highest carotenoids, which was not significantly ($p > 0.05$) different from the 28-day interval, while the 14-day interval had the least (Figure 6A). The highest total phenolics (Figure 6B) and total flavonoids (Figure 6C) were recorded with the application of PA at 21-day interval, followed by the 28-day interval, while the 14-day interval had the least (Figure 6B,C). Total sugar content was significantly ($p < 0.05$) high with the application of PA at 28-day interval, followed by 21-day interval, and 14-day interval had the least (Figure 6D).

Chemical Composition of the Grape Pomace. Total carotenoids (Figure 7A) and total phenolics (Figure 7B) were significantly ($p < 0.05$) high with the application of PA at 28-day interval, followed by 14-day interval. Plants subjected to 21-day interval PA application had the least pomace carotenoids. Unlike total carotenoids, the total flavonoids was not significantly ($p > 0.05$) different among the three different PA application frequencies (Figure 7C).

However, a nonsignificant ($p > 0.05$) improvement was observed with 14-day interval PA application, followed by a 21-day interval, and the least was recorded with 28-day interval. The total sugar was significantly ($p < 0.05$) high with the application of PA at 21-day interval, followed by 14- and then 28-day intervals (Figure 7D).

Chemical Composition of the Grape Juice. There were no significant ($p > 0.05$) differences in carotenoid, total phenolics, and total flavonoid of the grape juice with the application of PA at different frequencies (Figure 8A−C).

However, for carotenoids and total phenolics, nonsignificant ($p > 0.05$) increments were observed with the application of PA at 21-day interval, followed by 28-day interval, and the least was observed with 14-day interval (Figure 8A,B). A nonsignificant ($p > 0.05$) high total flavonoids was observed with PA application at 28-day interval, followed by 14-day interval and the 21-day interval had the least (Figure 8C). The juice total sugar content was significantly ($p < 0.05$) high with the application of PA at 28-day intervals, followed by 14-day interval, and 21-day interval had the least (Figure 8D).

The grape yield has strong positive correlations with vine length ($r = 0.997$), juice volume ($r = 1.000$), juice weight ($r = 1.000$), press weight ($r = 1.000$), °Brix, salinity ($r = 0.929$), total dissolved solids ($r = 0.805$), titratable acidity ($r = 0.880$), total phenolics in grape plant leaves ($r = 0.940$), total phenolics in juice ($r = 0.911$), total flavonoids in grape plant leaves ($r = 0.904$), total carotenoid in grape plant leaves ($r = 0.921$), carotenoids in juice ($r = 0.881$), and total sugar in pomace ($r = 0.992$) and a moderate association with electrical conductivity ($r = 0.560$) (Figure 9).

On the other hand, the yield is strongly correlated negatively with leaf area, chlorophyll content (SPAD), maximum quantum efficiency (F_v/F_m), potential photosynthetic capacity (F_v/F_o), total phenolics in pomace, total flavonoids in juice, carotenoids in pomace, and total sugar in juice.

A standard two-dimensional (2-D) PCA biplot illustrates the connections among various variables (Figure 10).

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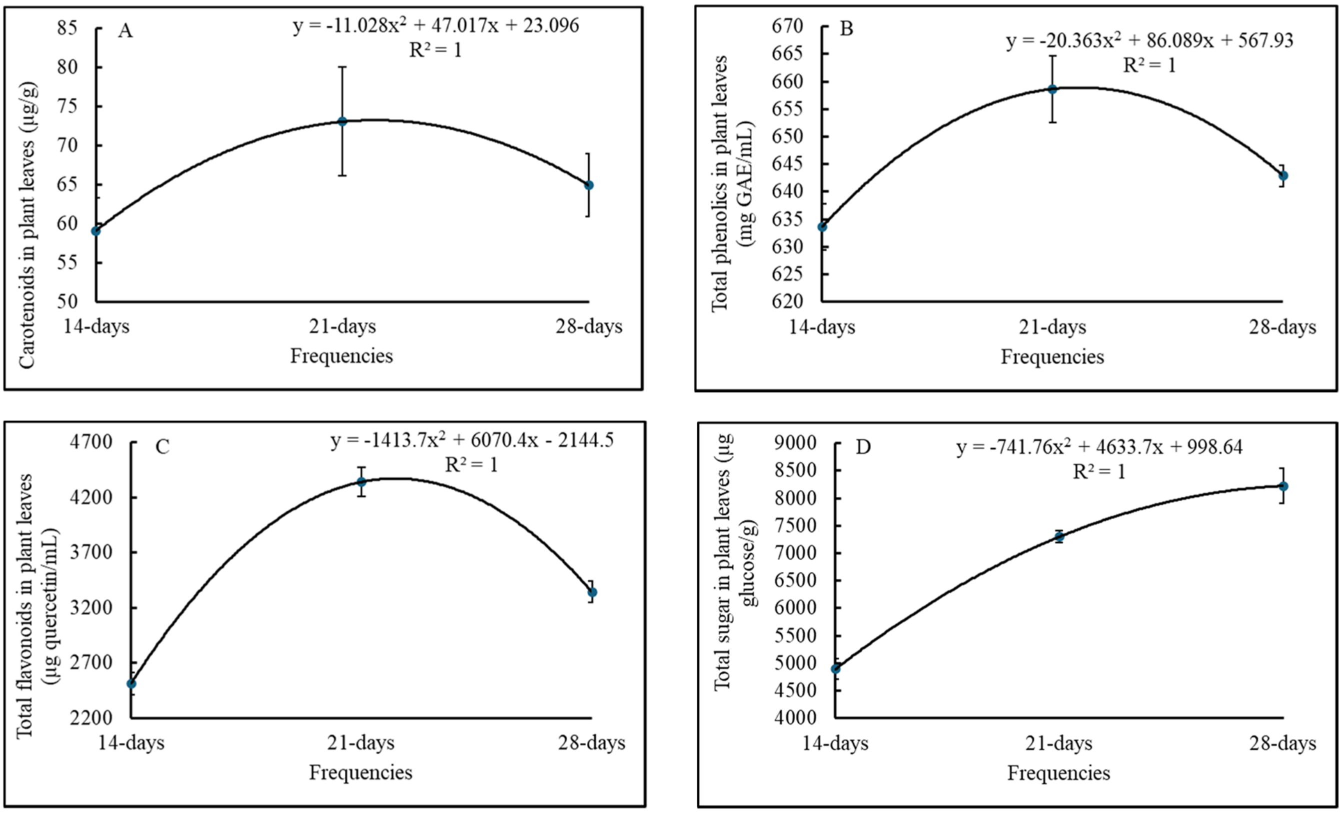

Figure 6. Grapevine response to varying frequencies of PA application. Change in (A) carotenoid, (B) total phenolics, (C) total flavonoid, and (D) total sugar in grape leaf following PA application at 14-, 21-, and 28-day intervals. Error bars represent standard deviation.

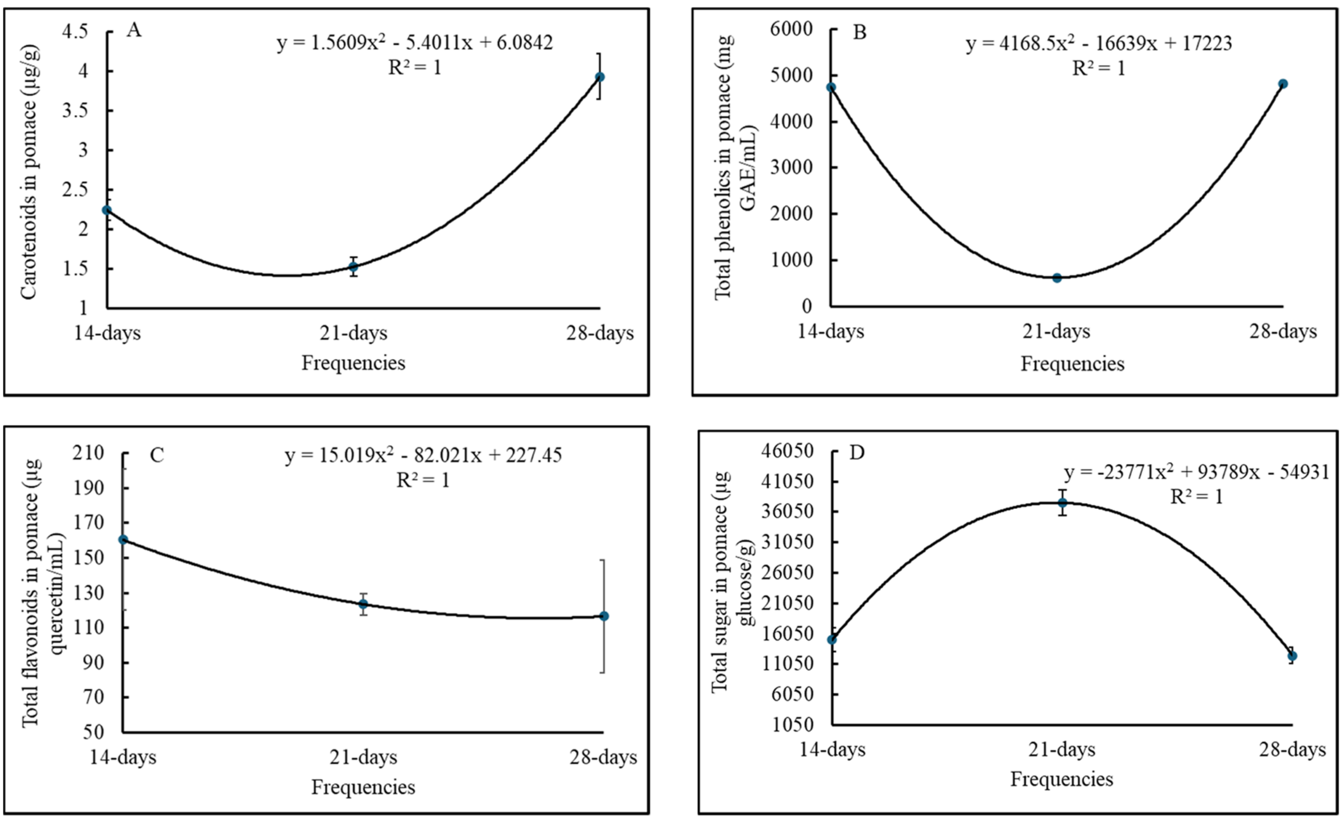

Figure 7. Grapevine response to varying frequencies of PA application. Change in (A) carotenoid, (B) total phenolics, (C) total flavonoid, and (D) total sugar in grape pomace following PA application at 14-, 21-, and 28-day intervals. Error bars represent standard deviation.

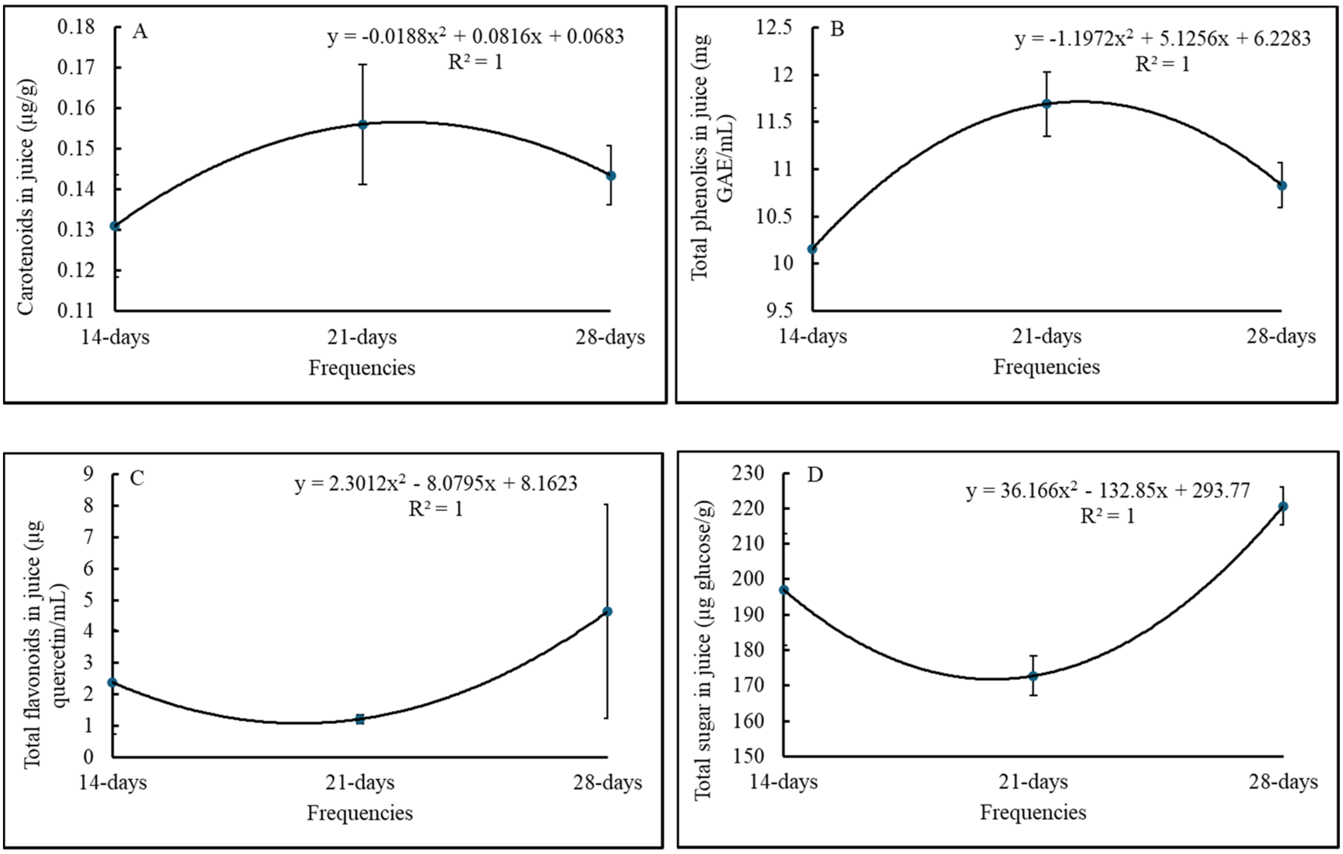

Figure 8. Grapevine response to varying frequencies of PA application. Change in (A) carotenoid, (B) total phenolics, (C) total flavonoid, and (D) total sugar in grape juice following PA application at 14-, 21-, and 28-day intervals. Error bars represent standard deviation.

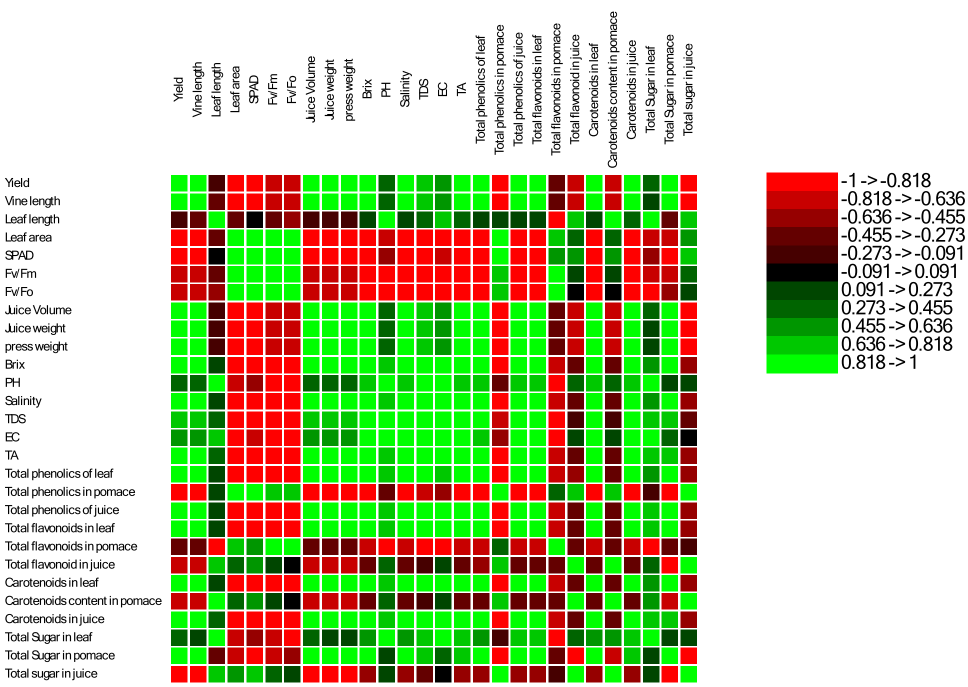

Figure 9. Heat map of grape plants response to pyroligneous acid applied at different frequencies correlation matrix between individual trait observed. The red color represents a strong negative association, and the green color represents a strong positive association.

The first and second principal components (PC1 and PC2) accounted for 76.09 and 23.91%, respectively, and thus explained about 100% of the total variations in the data set. The parameters that were grouped together indicated a positive correlation, whereas those that were separated suggested a negative correlation. Parameters positioned at right angles implied no correlation. Additionally, parameters clustered with a specific treatment indicated that the

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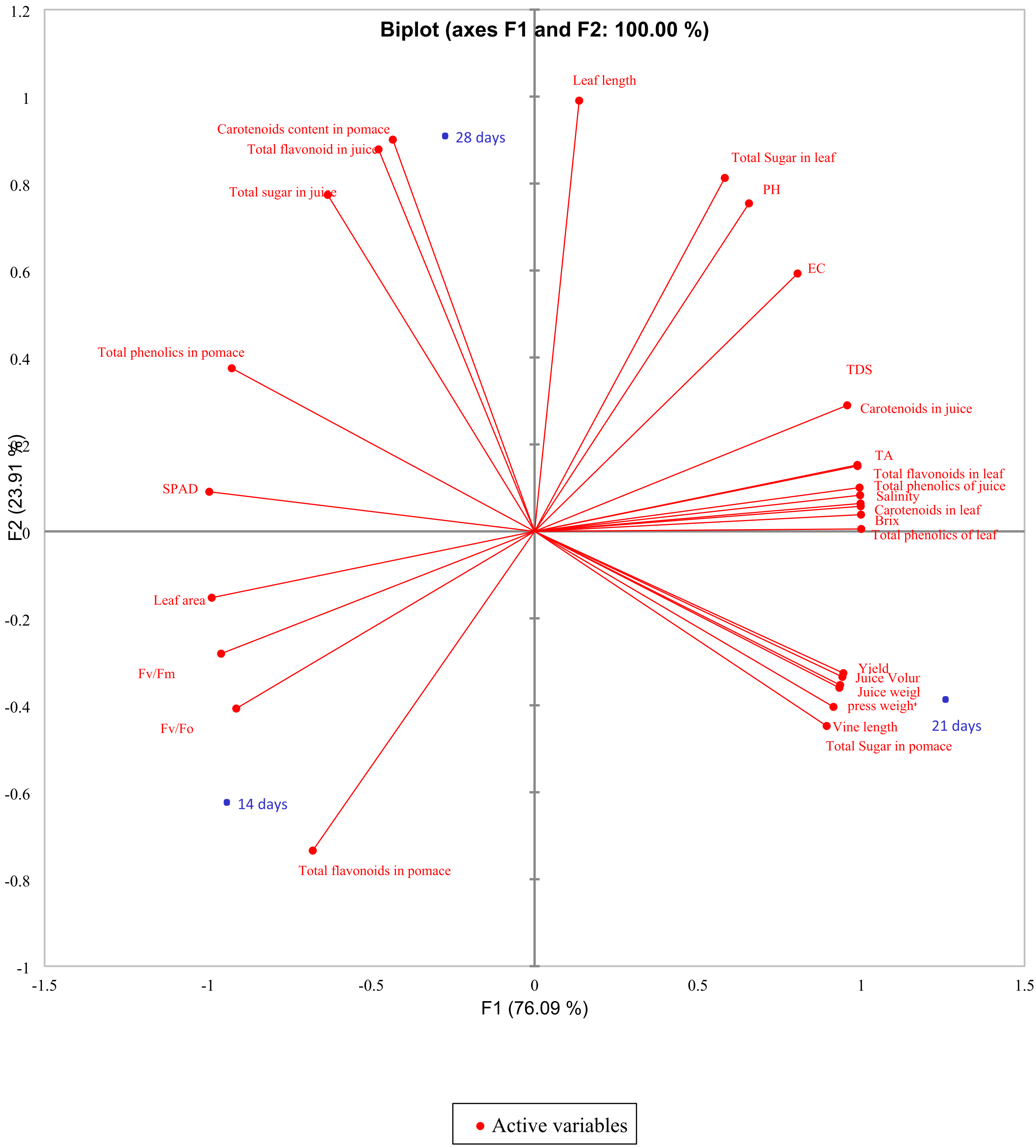

Figure 10. Two-dimensional principal component analysis (PCA) illustrating the relationships between various application frequencies of pyroligneous acid (PA) and the growth, yield, and quality characteristics of grape plants. The parameters examined include titratable acidity (TA), total dissolved solids (TDS), electrical conductivity (EC), maximum quantum efficiency (F_v/F_m), and photosynthetic capacity (F_v/F_o).

treatment had an effect on them, while those located further away from the treatment suggested a lack of influence from it. The PCA biplot showed that 4% PA applied at 14-day intervals influenced the grape plant leaf area, SPAD, maximum quantum efficiency (F_v/F_m), potential photosynthetic capacity (F_v/F_o), and total phenolics in pomace. The 21-day interval of PA application influenced vine growth, fruit fresh weight, juice weight, juice volume, press weight, °Brix, salinity, total dissolved solids, electrical conductivity, titratable acidity, total phenolics in grape plant leaves and juice, total flavonoids in grape plant leaves, total carotenoids in grape plant leaves and juice, and total sugar in the pomace. In addition, the 28-day interval of PA application influenced grape plant leaf growth, grape juice pH, total phenolics in pomace, total flavonoids in juice, carotenoids in pomace, and total sugar in grape plant leaves and juice.

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DISCUSSION

The application of biostimulants on grapevine is an effective and sustainable tool to enhance grape productivity, especially in response to the challenges posed by climate change in recent years.[45]

Applying biostimulants to grapevines during key phenological stages is essential for enhancing the grape yield. In the present study, we investigated the effects of pyroligneous acid on grapevine and leaf growth, yield, and chemical composition of the leaves, pomace, and juice over 2 years. PA, a naturally occurring compound, has been shown to enhance crop yields similar to synthetic plant growth regulators, while offering the advantage of being environmentally benign and sustainable.[13] Numerous studies indicated that the regulatory mechanisms and properties differ across various plant growth regulators.[46,47] In the present study, the 4% PA applied at 21- and 28-day intervals increased the growth of grapevine and leaves, respectively, without any stress on the plants. Dihydric phenols and polyphenols, which are phenolic compounds found in PA, have been shown to enhance plant growth when present in low concentrations.[13] The increase in the vine and leaf lengths with PA treatment can be linked to improved nutrient absorption and promotion of cell division and cell elongation, as previously explained in refs 48 and 21.

The composition of PA is intricate and made up of several bioactive compounds such as phenolics, alcohol, alkane, organic acid, and ester, thus making it challenging to elucidate its precise mode of action. The interactions between these compounds make it impossible to single out and attribute effects to just one component.[13,49] Therefore, due to the chemical composition of PA, different plant species respond differently to its application. The observed increase in grape yield linked to PA application is consistent with previous reports on rockmelon,[23] tomato,[14] and peanut.[50] In the present study, the application of 4% PA at 21-day intervals increased the grape berries yield. The decrease in yield observed in the present study as a result of small berry size with the application of the highest concentration of PA is also consistent with reports in refs 14 and 45. The application of PA at 21-day intervals to improve berry yield was also observed in ref 51. This suggests that the application of PA influences the absorption of essential nutrients, including calcium, magnesium, iron, nitrogen, phosphorus, potassium, and zinc for plant use causing changes in yield and yield parameters.[51]

Interestingly, the determinants of grape berry quality, such as °Brix, pH, salinity, total dissolved solids (TDS), electrical conductivity (EC), titratable acidity (TA), carotenoid, phenolics, and flavonoids, were improved by 4% PA applied at 21-day intervals. This indicates that PA has the potential to improve crop quality, which will benefit human health and nutrition. The findings agree with the reports in refs 14,23,48,51. The only differences in these reports could be the concentrations used, time of application, and the plant species used.

Generally, grapes are a good source of diverse phytochemicals and can be found in berry, seed, pomace, root, stem, cane, leaf, and skin. The significant compounds found are phenolic compounds, flavonoids, carotenoids, aromatic acids, and stilbenoids.[52−54] Nutritional constituents are also found in grapes apart from the bioactive compounds, and they include sugar, fibers, proteins, vitamin C, and minerals.[55−57] The polyphenols have the chemical structure and ability to act as radical scavengers of the lipid peroxidation chain reactions, donating electrons, and neutralizing free radicals.[58] These polyphenols were improved by PA application in this study, which may enhance the quality of the treated grape berries. These improved polyphenols can enhance the nutritional and health benefits of the consumers of the grapes obtained from the treated plants.

In the present study, PA increased the carotenoids, total phenolics, and total flavonoids but decreased the sugar content in the grapes, and these findings agree with a study on tomato in refs 14,51. Carotenoids are lipophilic compounds with a wide range of functions, especially beneficial to optical health.[59] There are 16 distinct classes of phenolic compounds, but the key ones relevant to human nutrition are phenolic acids, flavonoids, lignans, stilbenoids, and tannins.[60] Phenolic compounds represent the most prevalent class of chemical substances found in both dietary and medicinal plants.[61] According to ref 62, phenolic compounds are the essential contributors to the antioxidant capacity of dietary plants. Therefore, quantifying phenolic compounds in plants is crucial for selecting and developing cultivars with desirable traits.[63] Phenolic compounds offer a range of health benefits, including neuroprotection, antibacterial properties, antiaging effects, antioxidant activity, anti-inflammatory responses, antihyperlipidemic effects, cancer prevention, diabetes management, and cardiovascular protection.[60,64] Flavonoids are the largest group of phenolic compounds and can be found in many fruits.[65] Flavonoids are subgrouped into flavonols, flavanols, isoflavones, flavanones, flavone, and anthocyanin.[34] They are good sources of antioxidant and help in preventing cancer and heart diseases.[34] Sugar is a carbohydrate, and its quantity in plant products like fruits, leaves, and tubers determines the organoleptic qualities like taste of their produce.[27] The amount of alcohol content in wine, for instance, depends on the sugar level in the berries used in the production of wine.[32,33] In this study, these compounds were greatly improved by PA application; hence, this will aid solve many health problems and also contribute to the quality of foods.

In conclusion, PA use at different dosages and application intervals represents an innovative approach in viticulture. The application of 4% PA improved the berry fresh weight, total carotenoids, total phenolics, and flavonoids of the grape juice. Grape farmers, therefore, can adopt this concentration of PA application to enhance the yield and quality of their produce. PA applied at 21-day intervals improved vine growth, fruit fresh weight, juice weight, juice volume, press weight, °Brix, salinity, total dissolved solids, electrical conductivity, titratable acidity, total phenolics in grape plant leaves and juice, total flavonoids in grape plant leaves, total carotenoids in grape plant leaves and juice, and total sugar in the pomace in this present study. This could be good news for grape farmers to use fewer inputs in producing quality grapes. The low concentration and long application intervals of PA observed in this study suggest that farmers will reduce their cost of production and, hence, maximize their profits. Further study should be conducted to evaluate the effect of PA on metabolites in grape wine.

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Notes

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ABBREVIATIONS

PA:pyroligneous acid
Cv:cultivar
RCBD:randomized complete block design
N:number
USA:United States of America
pH:potential of hydrogen
TDS:total dissolved solids
EC:electrical conductivity
NaOH:sodium hydroxide
TA:titratable acidity
°C:degree celsus
mL:milliliter
g:gram
%:percent
min:minute
μL:microliter
nm:nanometer
UK:United Kingdom
μg:microgram
FW:fresh weight
TPC:total phenolics content
h:hour
mM:millimole
ANOVA:analysis of variance
LSD:least significant difference
PCA:principal component analysis

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