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Quantitative variation of phenolic compounds in different tissues of pistachio (*Pistacia vera* L. cv. Uzun) associated with alternate bearing

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Abstract: Alternate bearing is a common challenge in horticultural plants, leading to irregular yield in successive years. The potential role of phenolic compounds in regulating this phenomenon, however, is not well understood. This study aimed to investigate the potential role of phenolic compounds in alternate bearing in pistachio trees by analysing samples from different parts of the "Uzun" cultivar over two consecutive years. Seven phenolic compounds (gallic acid, p-coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, catechin, and quercetin) were analysed using high-performance liquid chromatography at 10-day intervals. Significant variations were observed in the levels of certain phenolic compounds between "ON" and "OFF" years, suggesting a potential role for these compounds in alternate bearing. Ferulic acid exhibited a significant decrease in leaves and shoots, indicating its translocation to the nuts, leading to a sharp decline during the nut lignification process. A negative and significant correlation between ferulic and caffeic acid levels was observed in the "ON" and "OFF" years, which may be linked to the alternate bearing and kernel development process in pistachio. These findings provide valuable insights into the role of phenolic compounds in regulating alternate bearing in pistachio trees and could inform future strategies for enhancing pistachio yields and quality.

Key words: *Pistacia vera* L., phenolic compounds, alternate bearing, principal component analysis (PCA)

1. Introduction

Pistachio (*Pistacia vera* L.) is the most economically significant cultivated species within the genus *Pistacia*, which belongs to the family Anacardiaceae and the order Sapindales (Ferguson and Polito, 2016). The pistachio tree thrives in dry, hot areas and can tolerate saline conditions (Kashani Nejad et al., 2003; Gündeşli et al., 2020). Turkey, a primary origin of pistachio, has valuable genetic sources and serves as a centre for the development and evolution of new pistachio varieties (Gündeşli et al., 2019). However, alternate bearing remains a significant issue that affects pistachio production, leading to irregular fruit yields across different years. This phenomenon occurs when the tree sheds inflorescence buds in an abundant year that would have produced the next year's crop, leading to inconsistent yields. Despite numerous efforts to explain this unusual phenomenon, the underlying mechanisms remain unknown. Consequently, alternate bearing negatively impacts consumers, producers, and the economy of the country (Hormaza and Wünsch, 2007; Okay et al., 2011; Goldschmidt, 2013; Gündeşli, 2020; Khezri et al., 2020).

Some studies suggest that alternate bearing in pistachio trees is influenced by not only genetic factors but also environmental and physiological factors, cultural management practices (Esmaeilpour and Khezri, 2006), tree nutrient balance (Baninasab and Rahemi, 2006; Baninasab et al., 2007; Güneş et al., 2010; Talaie et al., 2010; Marino et al., 2018), cultivar selection (Kallsen et al., 2007; Rosenstock et al., 2010; Vemmos, 2010), rootstock (Ferguson and Polito, 2016), and plant growth regulators (Lovatt and Ferguson, 2001; Okay et al., 2011; Gundesli et al., 2020; Khezri et al., 2020). However, little research has been conducted on the role of phenolic compounds in alternate bearing. Phenolic compounds are a subgroup of secondary metabolites with physiological and metabolic importance in plants (Shi et al., 2017). They play a crucial role in plant growth and reproduction, particularly in the defence mechanisms (Bravo, 1998). Additionally, phenolic compounds influence the taste of horticultural crops, including pistachios, which are a rich source of various polyphenolic compounds (Tokuşoğlu et al., 2005; Bodoira

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et al., 2019). Tomaino et al. (2010) identified 17 phenolic and polyphenolic compounds in pistachio nut and skin extracts, while Erşan et al. (2016) reported several phenolic compounds, including gallic acid, monogalloyl glucoside, and quercetin, at different ripening stages. However, the content of phenolic compounds in pistachio trees is influenced by environmental conditions and cultivar genotype (Colaric et al., 2005; Martínez et al., 2016) and changes throughout the growing season (Solar et al., 2006). Despite various studies on the phenolic content of nuts (Kornsteiner et al., 2006; Arcan and Yemenicioğlu, 2009), few studies have investigated the levels of phenolic compounds in pistachio organs other than the nut, and no study has examined their role in alternate bearing. Lavee et al. (1986) reported that phenolic acids, such as chlorogenic acid, ferulic acid, cinnamic acid, and caffeic acid, play a significant role in controlling the alternate bearing of olive trees. To the best of our knowledge, no studies have investigated the roles of phenolic compounds in the alternate bearing of pistachios across different growth periods and organs. Therefore, this study focuses on identifying the phenolic compounds in different organs, such as shoots, leaves, panicles, and nuts, of the "Uzun" pistachio cultivar. The objectives of this study were as follows: 1) to identify the phenolic compounds present in the different organs of the "Uzun" pistachio cultivar, 2) to investigate changes in the levels of phenolic compounds in relation to alternate bearing, and 3) to examine the relationship between these changes and both flower bud formation and kernel development stages.

2. Materials and methods

2.1. Plant materials

Samples were collected from six 34-year-old *Pistacia vera* cv., "Uzun" trees grafted on *P. vera* rootstocks (Table 1). The trees selected for this study were sourced from the experimental region of Dr. Ahmet Münir Bilgen at the Republic of Türkiye Ministry of Agriculture and Forestry Pistachio Research Institute. Known for its exceptional aroma and flavour, the "Uzun" cultivar holds significant value in Türkiye. It is characterized by a sturdy, semiupright tree structure and is classified as a midflowering cultivar.

In 2013, a total of 40 trees were selected as control trees for a comprehensive evaluation of the ON year and OFF year trees in their natural bearing status. Among the OFF year trees, some buds were artificially removed, and this process was repeated until the sampling year of 2015 and 2016 to gain a complete understanding of the alternate bearing mechanism. Throughout the growing years of 2015 and 2016, samples of leaves, shoots, peduncles, and fruits were collected at 10-day intervals, beginning approximately 45 days after full bloom (from early May to mid-July). Phenology of the trees was observed to determine the time of full bloom in the selected trees. Additionally, climate data for the region, obtained from the General Directorate of Meteorology, were analysed to provide a better understanding of the results (Figure 1).

2.2. Biochemical analysis

Shoot, leaf, nuts and peduncles collected from selected trees were sampled early in the morning and immediately transferred to the laboratory under cold chain conditions. They were then dried in a lyophiliser for one week.

2.2.1. Detection of phenolic compounds

2.2.2. Sample preparation

The phenolic compounds in the samples were extracted using 10 mL of a 25% methanol solution containing 100 µL of trifluoroacetic acid, after refluxing at 100 °C for 1 h. The resulting extract was then adjusted to a final volume of 10 mL and centrifuged at 4500 rpm for 15 min. The supernatant was filtered through a 0.2 µm filter and subsequently analysed using HPLC.

Table 1. Sampling dates of biochemical analyses.

2015 Sampling dates		2016 Sampling dates	
-	-	15 DAFB	22 April 2016
-	-	22 DAFB	29 April 2016
35 DAFB	15 May 2015	29 DAFB	06 May 2016
45 DAFB	25 May 2015	36 DAFB	13 May 2016
55 DAFB	04 June 2015	50 DAFB	27May 2016
65 DAFB	14 June 2015	64 DAFB	10 June 2016
75 DAFB	24 June 2015	78 DAFB	24 June 2016
86 DAFB	05 July 2015	92 DAFB	08 July 2016
118 DAFB	06 August 2015	127 DAFB	12 August 2016
146 DAFB	03 September 2015	147 DAFB	01 September 2016

*DAFB = days after full bloom.

*The full bloom dates in the study were 10 April 2015 and 08 April 2016, respectively.

Figure 1. Metrological data of the experiment field for the 2015 and 2016 years.

2.2.3. HPLC analysis of phenolic compounds

Using the modified method of Kosar et al. (2004), high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan) equipped with a photodiode array detector (DAD) was used to detect and quantify seven specific phenolic compounds: gallic acid, p-coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, catechin, and quercetin. An analytical column of Inertsil ODS-3 (C18) with a particle size of 5 µm, a diameter of 4.6 mm and a length of 250 mm was used for the separation of the phenolic compounds. The column was operated at a temperature of 40 °C, with a flow rate of 1 mL/min. The compounds were detected in the wavelength range of 280 nm to 360 nm. Elution was performed with a nonlinear gradient of a solvent mixture comprising 2.5% formic acid in water (solvent A) and 2.5% formic acid in acetonitrile (solvent B). The proportion of solvent B was gradually increased during the analysis: starting at 5%, it increased to 13% in 15 min, then to 15% in 5 min, followed by a rise to 30% in another 5 min, which was maintained for 3 min. Subsequently, gradual increase was observed, reaching 45% in 4 min, which was then maintained for 3 min. This was followed by a further increase to 90% in 5 min,, which was also maintained for 5 min before returning to baseline conditions within 5 min. To identify the components, their retention times were compared with those of the external standards used in the analysis. All the standards we utilized were sourced from Sigma-Aldrich and Merck, each with a purity exceeding 98.0%, and they were labelled as analytical standards.

2.3. Statistical analysis

For the statistical analysis of the results obtained, the principal component analysis (PCA), Mann–Whitney U test, t-test, and logit regression were used. Mann–Whitney U test was employed to investigate whether the phenolic levels in different organs had statistical significance in ON and OFF trees. This nonparametric method was chosen because the number of observations was low for the different organs and the distribution of data did not meet the normality condition. SPSS program, XLSTAT, and STATA software were used for Mann–Whitney U test, PCA and t-test, and regression analysis, respectively.

3. Results

Recent biochemical studies have highlighted the crucial role of phenolic compounds in enhancing the nutritional value and health benefits of fruits. In this study, the primary objective of the study is to investigate the impact of phenolic compounds on the alternate bearing of the "Uzun" pistachio variety (*Pistacia vera* L. cv. Uzun) across various tissues. Additionally, the study aims to determine the concentration of these compounds to evaluate their potential in promoting the healthy aspects of pistachio.

The phenological observations indicated that full bloom occurred on 08 April 2015 and 10 April 2016. Despite the same temperature changes in both years, the higher humidity levels in 2015 may have led to earlier blooming compared to 2016.

3.1. Phenolic compounds concentrations (µg/g) and changes pattern in leaves

The significant differences ($p < 0.05$) in the phenolic compounds (µg/g) of "ON" and "OFF" leaves were recorded (Tables 2–4). Analysis of the leaves revealed that ferulic acid was the most abundant phenolic compound. The highest concentrations of ferulic acid were found in 2015 and 2016, during the "ON" years, with values of 1134.97 µg/g and 960.52 µg/g, respectively (Figure 2). Based on the findings, the content of ferulic acid in the leaves exhibited a pattern of high concentration at the start of the season, followed by a gradual decrease throughout both "ON" and "OFF" years. The levels of ferulic acid continued to decline as the season progressed, reaching their lowest point during the harvest period in both years. The results showed that the accumulation of ferulic acid during the lignification period of nut development was significantly higher in the "ON" year than in the "OFF" year. In the "ON" years, the amount of caffeic acid was higher compared to the "OFF" years. The observed variation in caffeic acid content ranged from 15.56 µg/g to 30.78 µg/g over the two years of the study. Conversely, during the "OFF" year, the range of caffeic acid content was between 17.70 and 43.57 µg/g in 2015 and between 17.84 and 30.62 µg/g in 2016. The caffeic acid contents in the leaves of "OFF" trees were significantly lower than in "ON" trees until 36 DAFB. Thereafter, the caffeic acid concentration increased sharply in "OFF" trees while also increasing in "ON" trees,

Table 2. t-test results for data from the year 2015.

Phenolic	ON/OFF	Observations	Mean ± SD	t	p-value
CA	ON	32	14.76 ± 8.51	2.459	0.0178
	OFF	16	22.16 ± 12.12		
GA	ON	32	18.61 ± 12.48	1.4983	0.1409
	OFF	16	24.41 ± 13.00		
p-CA	ON	32	11.92 ± 1.91	–0.4012	0.6902
	OFF	16	11.71 ± 1.04		
ChA	ON	32	34.23 ± 25.5	0.2257	0.8224
	OFF	16	35.77 ± 12.9		
CT	ON	32	113.34 ± 63.35	–2.3536	0.0229
	OFF	16	72.00 ± 42.39		
FA	ON	32	253.94 ± 335.19	1.4866	0.1439
	OFF	16	415.27 ± 391.18		
QN	ON	32	37.83 ± 34.26	0.1481	0.8829
	OFF	16	39.43 ± 37.34		

*Caffeic acid (CA), gallic acid (GA), p-cumaric acid (p-CA), chlorogenic acid (ChA), catechin (CT), ferulic acid (FA), quercetin (QN).

Table 3. t-test results for data from the year 2016.

Phenolic	ON/OFF	Observations	Mean ± SD	t	p-value
CA	ON	38	10.93 ± 7.81	2.6593	0.0102
	OFF	20	16.69 ± 7.89		
GA	ON	38	21.85 ± 18.66	1.3704	0.176
	OFF	20	29.61 ± 23.68		
p-CA	ON	38	11.98 ± 1.59	–0.8095	0.4217
	OFF	20	11.67 ± 0.88		
ChA	ON	38	30.54 ± 14.34	0.5904	0.5573
	OFF	20	32.76 ± 12.05		
CT	ON	38	71.46 ± 29.88	–0.9094	0.367
	OFF	20	64.16 ± 27.4		
FA	ON	38	235.24 ± 314.98	1.3768	0.1741
	OFF	20	369.23 ± 415.42		
QN	ON	38	37.95 ± 28.16	0.7355	0.4651
	OFF	20	44.69 ± 41.22		

*Caffeic acid (CA), gallic acid (GA), p-cumaric acid (p-CA), chlorogenic acid (ChA), catechin (CT), ferulic acid (FA), quercetin (QN).

Table 4. Pooled logit regression analysis for the year 2015.

R.E.F. "ON" × "OFF"	CA	GA	p-CA	ChA	CT	FA	QN
CA	−0.0814* (−2.32)						
GA		−0.0417 (−1.56)					
p-CA			0.0933 (0.43)				
ChA				−0.00347			
CT					0.0202* (−2.24)		
FA						−0.00128 (−1.48)	
QN							−0.00144 (−0.16)
β_0	1.752 (1.73)	1.878 (1.56)	−0.371 (−0.14)	0.790 (0.83)	−0.763 (−0.69)	1.208 (1.25)	0.765 (0.78)
N	48	48	48	48	48	48	48

R.E.F. Regression estimated for statistics in parentheses; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; (β_0) intercept term in the regression model. Caffeic acid (CA), gallic acid (GA), p-cumaric acid (p-CA), chlorogenic acid (ChA), catechin (CT), ferulic acid (FA), quercetin (QN).

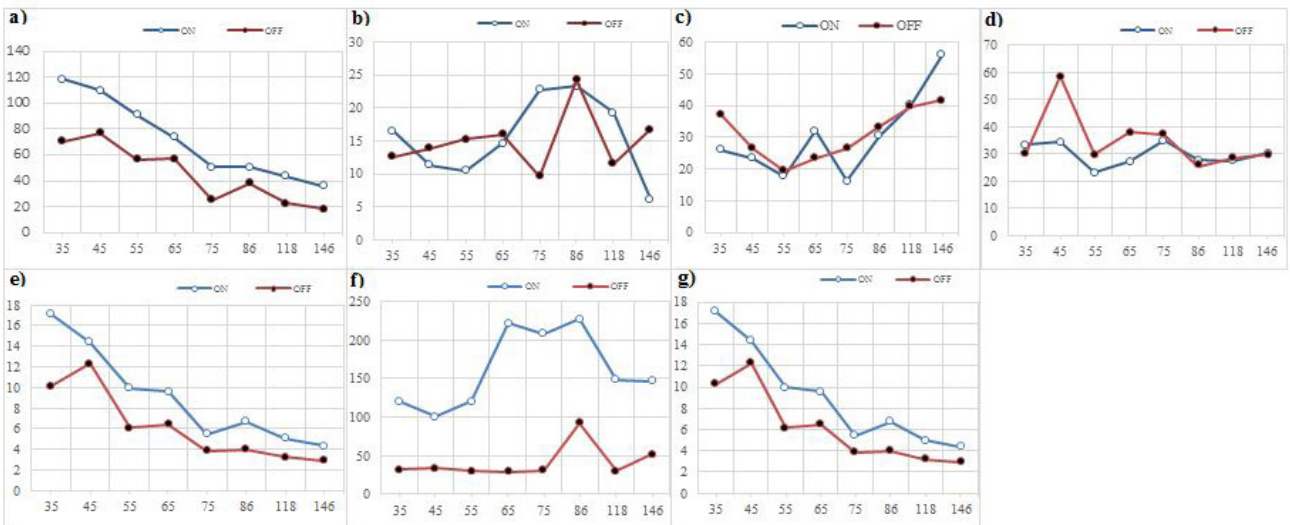

Figure 2. Variations of a) ferulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in leaves of "OFF" and "ON" trees in the year 2015. y-axis: date after full bloom; x-axis: concentration in (μg/g).

indicating that a critic period between "ON" and "OFF" years during 50–92 DAFB in 2015 (Figure 2) and 55–118 DAFB in 2016 (Figure 3), which coincided with the second phase of nut development. The seasonal variations of caffeic acid in "OFF" trees were significant. The analysis revealed that the concentration of chlorogenic acid in the leaves of "OFF" year trees was higher than that of "ON" year trees, particularly during two phases of nut development in June and August (Figure 3). The amount of chlorogenic acid varied between 20.41 and 45.32 μg/g across the two years of the study (Figure 3). The concentration of gallic acid ranged from 1.97 to 20.26 μg/g. In both years of the study, a gradual decrease in gallic acid concentration was observed during 75 DAFB period in 2015 and 64 DAFB in 2016, in both "ON" and "OFF" years, which coincides with the first phase of bud abscission. However, the amount of

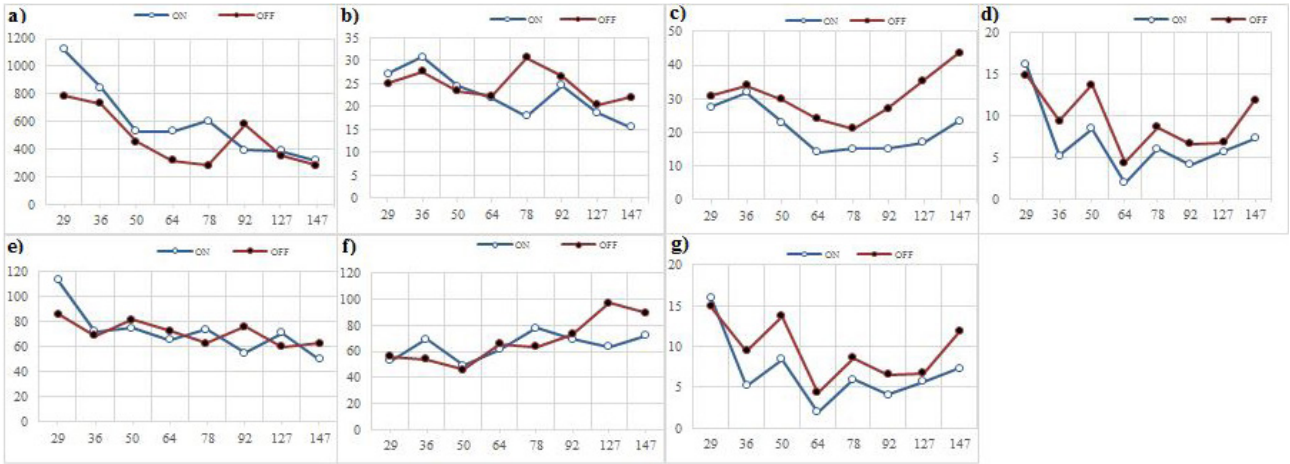

Figure 3. Variations of a) f erulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in leaves of "OFF" and "ON" trees in the year 2016. y-axis: date after full bloom; x-axis: concentration in (µg/g).

gallic acid in the "ON" years was significantly lower than in the "OFF" years. Quercetin was the second major phenolic compound in the leaves, with concentrations ranging between 49.82 and 113.45 µg/g in the studied samples. In the "ON" years, the amount of quercetin was slightly higher than in the "OFF" years, particularly in 2015. In the early season, the concentration of quercetin was high and subsequently decreased to a minimum in the "OFF" year. The concentration of catechin in the leaves was initially low at the beginning of the season in both bearing and nonbearing trees. However, as the season progressed, the concentration of catechin increased in both types of trees. In "OFF" year trees, there was a decreasing trend in catechin concentration towards the end of the season. However, approximately 120 DAFB, there was a sudden increase in catechin concentration in "OFF" year trees. No significant difference was found in the p-coumaric acid concentration between bearing and nonbearing, and its content ranged from 10.61 to 19.71 µg/g in all tested samples (Figure 3).

3.2. Phenolic compounds concentrations (µg/g) and changes pattern in shoots

Similar to the leaves, ferulic acid was the predominant phenolic compound in the shoots. The amount of ferulic acid varied between 28.30–190.53 and 17.85–293.20 µg/g in "ON" and "OFF" years, respectively. The amount of ferulic acid gradually declined in both 2015 and 2016, from a high level at the beginning of the season to a minimal level at harvest time (Figures 4). The amount of caffeic acid showed significant fluctuations both across different bearing years and within the studied years. Its concentration varied between 10.47–23.34 and 5.08–12.30 µg/g in 2015 and 2016, respectively, during the "ON" year, and between 1.19–24.23 and 5.78–14.95 µg/g in 2015 and 2016, respectively, during the "OFF" year. The amount of chlorogenic acid varied between 16.31 and 70.99 µg/g among the studied samples. The concentration of chlorogenic acid was found to be high in both "ON" and "OFF" year shoots in May. However, it decreased 36 DAFB and exhibited a gradual increase thereafter until harvest time. During harvest time, an increase in the concentration of chlorogenic acid was observed in "ON" year shoots, while in "OFF" year shoots, the concentration of chlorogenic acid tended to decrease. Generally, the concentration of gallic acid was higher in the "OFF" years than in the "ON" years. However, its concentration decreased gradually in the "OFF" years throughout the season, except for a sudden increase in mid-June. The amount of gallic acid reached a minimum level during June, coinciding with a period of intensive bud abscission. The shoot samples under study showed a quercetin concentration ranging from23.07 to 98.95 µg/g. In 2016, the amount of quercetin was higher in "OFF" year trees, whereas in 2015, the opposite was observed, with higher amounts found in "ON" year trees.

At the beginning of the season, the concentration of quercetin was at its maximum level but then decreased rapidly, reaching a minimum level at 45 and 29 days after full bloom in 2015 and 2016, respectively. The amount of quercetin then remained steadily low until the harvest time, ranging from 2.16 to 21.96 µg/g. The concentration of catechin, the second major phenolic compound in shoots, ranged from 29.16 to 227.89 µg/g in the studied samples. Specifically, the concentration of catechin was significantly higher in 2015 compared to 2016. Additionally, the variations between "ON" and "OFF" years were also distinctly observed in both years. The amount of p-coumaric acid varied between 9.52 and 14.05 µg/g among all samples. No differences were detected in p-coumaric acid concentration between "ON" and "OFF" years, and similar changes were recorded during most periods (Figures 4 and 5).

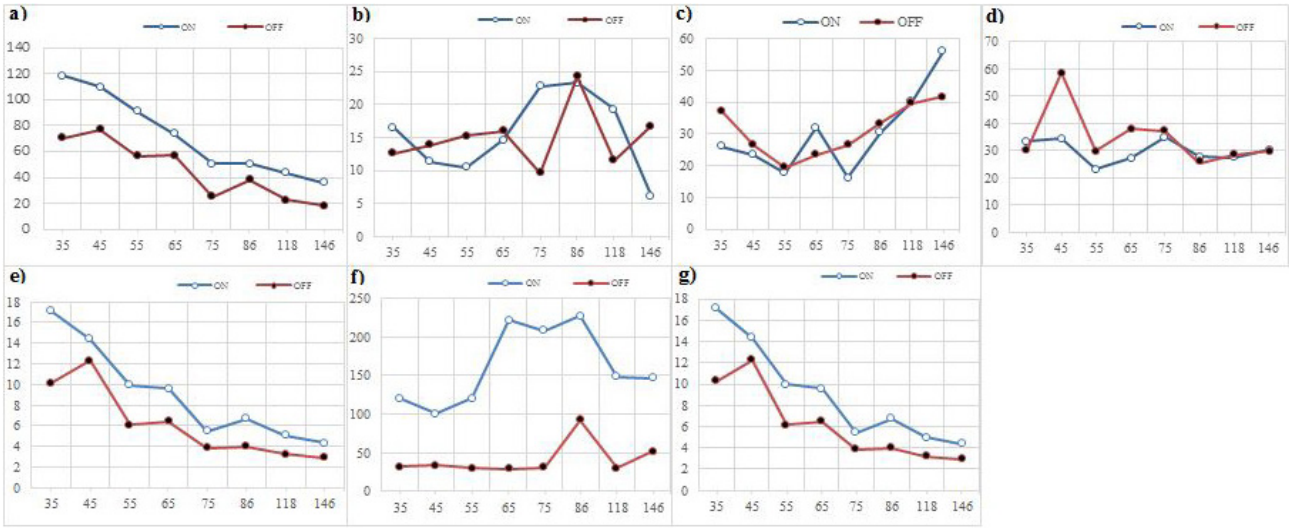

Figure 4. Variations of a) ferulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in shoots of "OFF" and "ON" trees in the year 2015. y-axis: date after full bloom; x-axis: concentration in (µg/g).

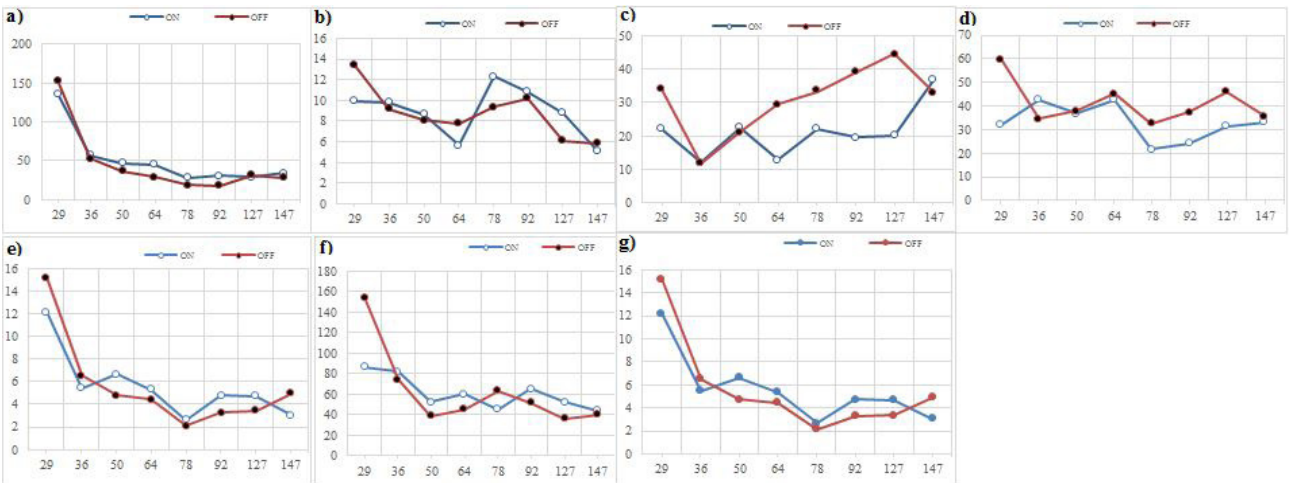

Figure 5. Variations of a) ferulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in shoots of "OFF" and "ON" trees in the year 2016. y-axis: date after full bloom; x-axis: concentration in (µg/g).

3.3. Phenolic compounds concentrations (µg/g) and changes pattern in peduncles and nuts

The concentration of ferulic acid ranged from 16.41 to 80.01 µg/g in peduncles and from 19.00 to 215.83 µg/g in nuts (Figure 6). In peduncles, the concentration of ferulic acid was high in May but decreased by late June. Conversely, the concentration of ferulic acid in nuts was high at the beginning of the season and then sharply declined during nut development, reaching its minimum level. The concentration of caffeic acid ranged from 2.51 to 14.00 µg/g in peduncle and from 2.12 to 8.22 µg/g in nuts in 2016 (Figure 7). Despite the low concentration at the beginning of the season, the concentration of caffeic acid increased in peduncles during fruit development. Conversely, the amount of caffeic acid in the nuts was high at beginning of the season, then decreased and remained at a low, constant level until May 2016. The amount of chlorogenic acid varied between 15.25 and 126.66 µg/g in peduncle and between 16.33 and 46.26 µg/g in nuts in 2015 (Figure 8). The concentration of chlorogenic acid in peduncles was high in May, decreased slightly in June, and then increased again near harvest time. Conversely, the concentration of chlorogenic acid in nuts was high in mid-June and early July, when bud abscission was intensive, which is contrary to the pattern observed in peduncles in 2015 (Figures 8). The concentration of gallic acid ranged from 4.74 to 14.46 µg/g in peduncle and from 1.37 to 57.97 µg/g in nuts in 2015. The high concentration of gallic acid decreased until

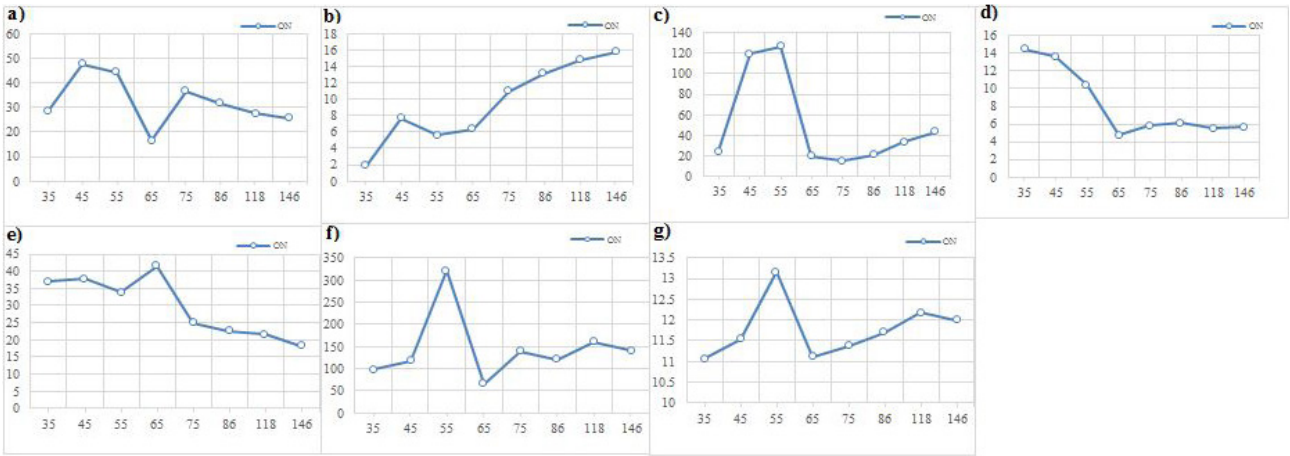

Figure 6. Variations of a) ferulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in peduncles "ON" trees in the year 2015. y-axis: date after full bloom; x-axis: concentration in (µg/g).

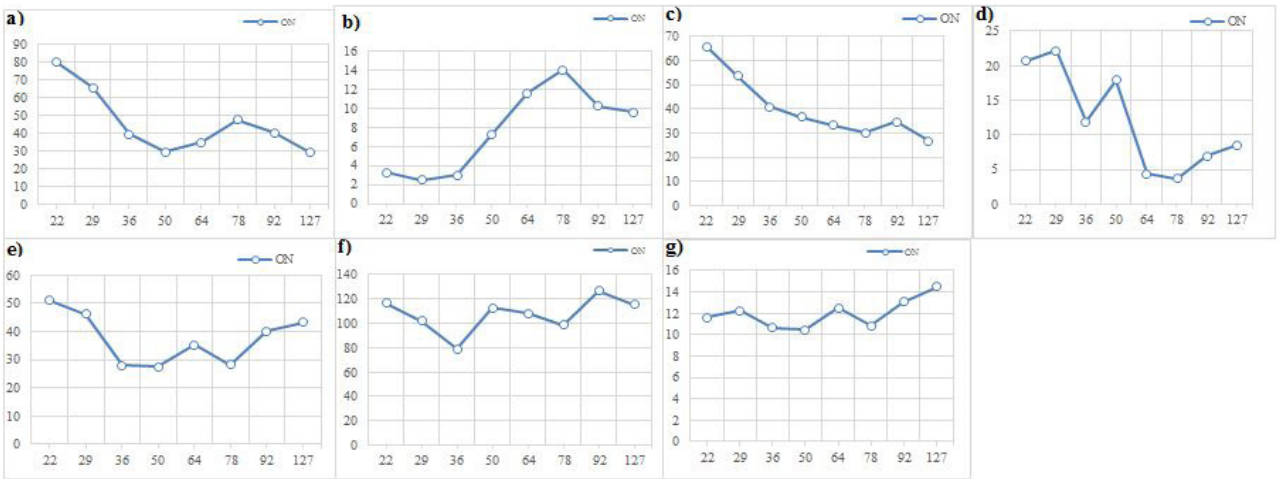

Figure 7. Variations of a) ferulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in peduncles of "ON" trees in the year 2016. y-axis: date after full bloom; x-axis: concentration in (µg/g).

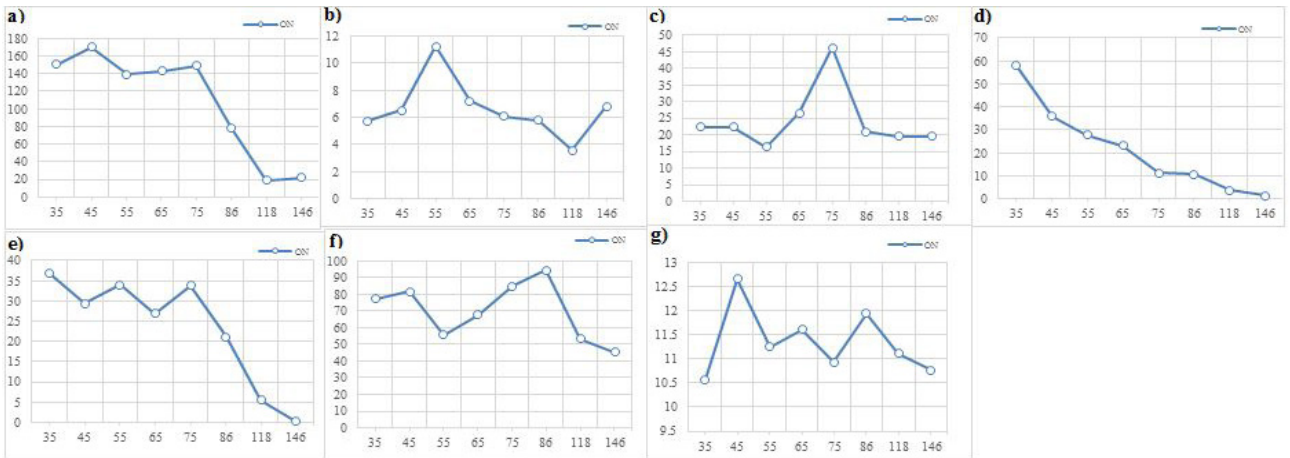

Figure 8. Variations of a) ferulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in nuts of "ON" trees in the year 2015. y-axis: date after full bloom; x-axis: concentration in (µg/g).

approximately 65 DAFB, the time of bud abscission, and then remained at that level throughout 2015. Similarly, in nuts, the concentration of gallic acid was initially high at the beginning of full bloom but gradually decreased as the season progressed, reaching a minimum near harvest time (Figure 8). The amount of quercetin varied between 18.13 and 41.61 µg/g in peduncles and between 0.32 and 36.76 µg/g in nuts in 2015. The concentration of quercetin in peduncles exhibited a significant decline in May, in contrast to its high levels at the beginning of the season. Although the amount of quercetin in nuts was also high in the early season, its concentration decreased during the bearing season and reached its minimum level near harvest (Figure 9). The amount of catechin varied between 65.97 and 323.11 µg/g in peduncle and between 45.42 and 94.45 µg/g in nuts in 2015. The catechin concentration of peduncle was low at approximately 35 DAFB and showed a peak at 55 DAFB, followed by a decrease. After an initial slight increase, a downward trend was observed until harvest time. Similarly, the catechin concentration in the nuts was high from mid-May until mid-June. However, it increased during the seed filling period, followed by a decrease at 65 DAFB (Figure 9). The concentration of p-coumaric acid ranged between 11.06 and 13.16 µg/g in peduncle and between 10.58 and 12.66 µg/g in nuts in 2015. In peduncles, the concentration of p-coumaric acid increased gradually throughout the season. In nuts, however, the amount of p-coumaric acid was high from 35 to 55 DAFB, then decreased, and increased again near harvest time (Figure 9).

3.4. Results of statistical analyses

3.4.1. t-test results

A t-test analysis was applied to all data to determine the most important variables explaining the relationships between the identified phenol compounds and alternate bearing to determine any group model (Tables 2 and 3). The results presented in Tables 2 and 3 represent the mean concentrations from triplicate analyses. When comparing "ON" and "OFF" years, caffeic acid, gallic acid, chlorogenic acid, ferulic acid, and quercetin levels were observed to be lower in the "ON" years than in the "OFF" years. p-coumaric acid and catechin levels were higher in the "ON" years. The largest fluctuation was observed in ferulic acid in both "ON" and "OFF" years. The fluctuations in caffeic, gallic, and ferulic acids, as well as quercetin, were lower in the "ON" years based on the sampling time. Caffeic acid and catechin levels were significantly different between "ON" and "OFF" years when considering all explant sources collectively (Tables 2 and 3). Caffeic acid was low, whereas catechin concentration was higher in the "ON" years (Table 3). The only difference between the two studied years in the t-test results was catechin, which was not significant in 2016.

3.4.2. Pooled logit regression analysis average for 2015 and 2016 data

Regression analyses are performed separately for all variables in Tables 4 and 5. Caffeic, gallic, chlorogenic, and ferulic acids, as well as quercetin, are significantly and negatively affected by trees in the "ON" year. Tables 4 and 5 display the correlation coefficients between phenolic compounds and the alternate bearing. Some features have been found to be strongly interrelated. Significant negative and positive correlations were observed for caffeic acid.

3.4.3. Principal component analysis

Principal component analysis (PCA) is a statistical technique used for dimensionality reduction and data visualisation. It provides a way to summarise the most important information in a multidimensional data Table.

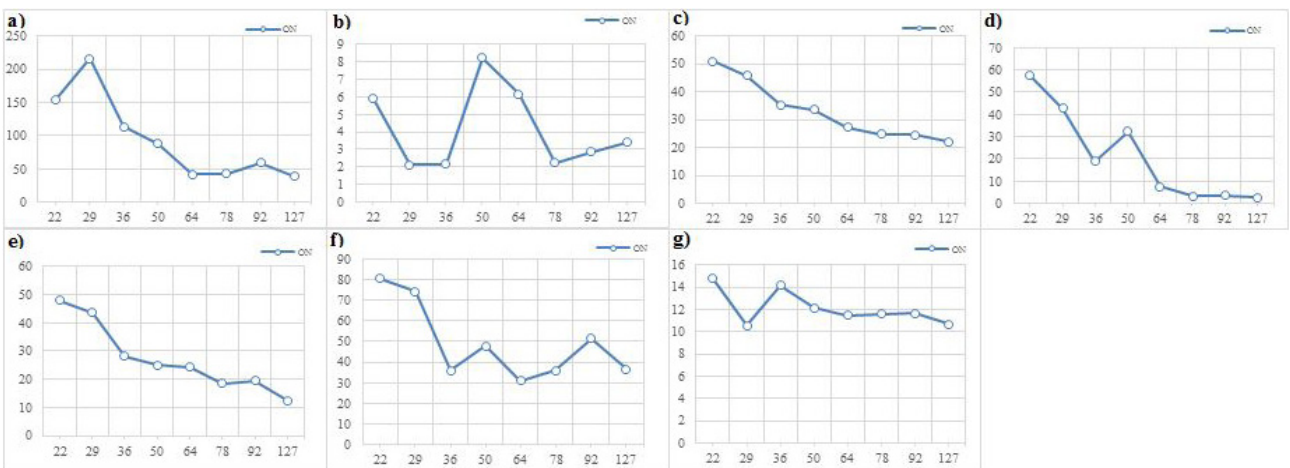

Figure 9. Variations of a) ferulic acid, b) caffeic acid, c) chlorogenic acid, d) gallic acid, e) quercetin, f) catechin, g) p-cumaric acid in nuts of "ON" trees in the year 2016. y-axis: date after full bloom; x-axis: concentration in (µg/g).

Table 5. Pooled logit regression analysis for the year 2016.

R.E.F. "ON" × "OFF"	CA	GA	p-CA	ChA	CT	FA	QN
CA	–0.0924* (–2.50)						
GA		–0.0262 (–1.60)					
p-CA			0.213 (0.89)				
ChA				–0.0176 (–0.68)			
CT					0.00997 (0.91)		
FA						–0.00134 (–1.55)	
QN							–0.00755 (–0.85)
β_0	1.852 (1.80)	2.003 (1.57)	–1.891 (–0.63)	1.592 (0.99)	–0.0696 (–0.06)	1.463 (1.38)	1.172 (1.11)
N	58	58	58	58	58	58	58

R.E.F. Regression estimated for statistics in parentheses; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; (β_0) intercept term in the regression model. Caffeic acid (CA), gallic acid (GA), p-cumaric acid (p-CA), chlorogenic acid (ChA), catechin (CT), ferulic acid (FA), quercetin (QN).

By plotting the principal components, we can visualise the relationships between different variables and gain insight into sampling patterns, groups, similarities or differences within the data (Kara, 2009). In this study, PCA was used to identify the major phenolic compounds contributing to the observed bud abscission patterns and to assess their relationship with other variables. This analysis provides a comprehensive understanding of the factors influencing alternate bearing in "Uzun" pistachio, allowing for a more informed interpretation of the data and potential insights into management strategies for regulating bud abscission. In 2015, PC1 and PC2 explained 46.59% and 22.07% of the total variance in nut samples, respectively. The variance of the two factors was 68.66% of the total variance. The first days (35, 65, and 45), except the 55th day, are the periods when phenolic levels are highest. Additionally, the first and the last days formed separate groups (Figure 10). In 2016, PC1 and PC2 explained 74.03% and 16.16% of the total variance, respectively. The variance of the two factors was 90.19% of the total variance. It appears that the first sampling days (14–22 DAFB) differ from the others, with day 29 showing the greatest difference. Additionally, it was observed that the phenolic levels were higher in the first days and decreased notably during the fruit filling period (Figure 10).

In 2015, PC1 explained 47.09% of the total variance, while PC2 explained 35.99% in peduncle samples. These two components collectively accounted for 83.08% of the total variance. Although there is no clear grouping, it is evident that caffeic acid levels have increased in recent days (Figure 10). In 2016, PC1 explained 56.10% of the total variance, PC2 explained 23.90%. These two components accounted for 80.00% of the total variance. Generally, it is seen that the first days form a group and the phenolic levels are higher during these days. The last days can be divided into two groups: 36, 50, and 78 form one group, while 64, 92, and 127 form the other group (Figure 10).

In 2015, PC1 and PC2 explained 37.48% and 26.13% of the total variance in leaf samples, respectively. These two factors accounted for 63.62% of the total variance. Caffeic acid and catechin levels increased in the "OFF" year, while p-coumaric acid, ferulic acid, and quercetin levels were higher in the "ON" year. Apart from these observations, there is no distinct grouping, although some differences were noted within the same period. When considered independently from bearing status, the first and the last days show a separation (Figure 11). In 2016, PC1 and PC2 explained 47.72% and 22.53% of the total variance, respectively. These two factors accounted for 71.25% of the total variance. Many phenolic compounds appear to be lower in the "ON" years and the last days of sampling dates. The amount of catechin is higher in the "OFF" year (sampling date 78-92-12), as well as on the last days. When considered independently of the ON-OFF years, it is observed that the first and the last days are distinct, with the phenolic amount being higher on the first days (Figure 11).

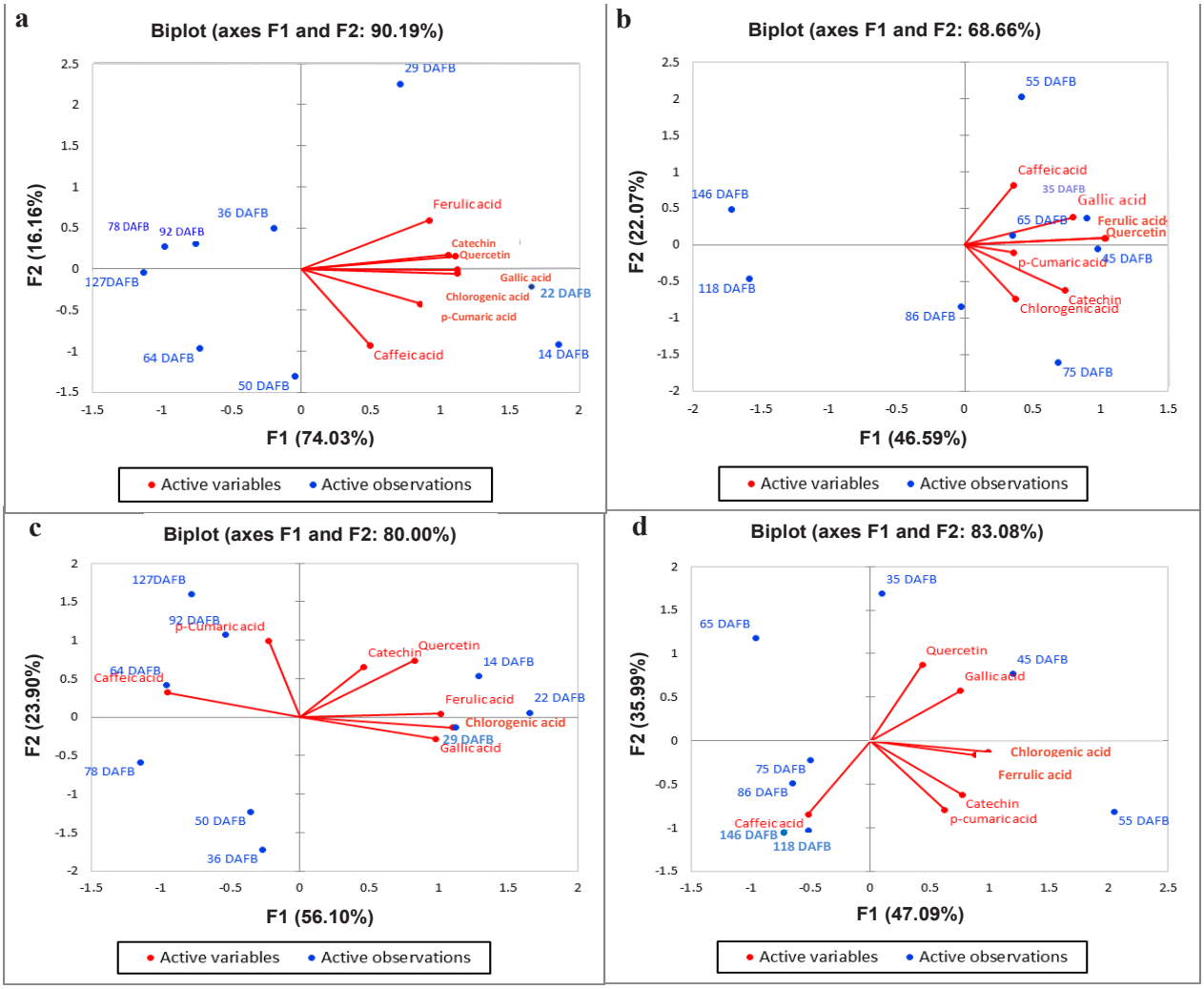

Figure 10. Biplot graph (scores and loading plots) obtained from principal component analysis, a) nut, 2016, b) nut, 2015, c) peduncle, 2016, d) peduncle, 2015.

In the 2015 shoot samples, PC1 explained 34.29% of the total variance, and PC2 explained 26.35%. They accounted for 60.63% of the total variance. Phenolic compounds (excluding chlorogenic acid) were found to be higher in the first days (Figure 11). In 2016, PC1 and PC2 explained 52.30% and 20.61% of the total variance, respectively. The variance that these two factors explain was 72.92% of the total variance. It was observed that phenolics (except p-coumaric) were higher during the first sampling periods in both the "ON" and "OFF" years, and the levels of these phenolics decreased significantly over time.

Some important differences between phenolic compounds and alternate bearing were confirmed by PCA analysis. Overall, the PCA results indicated a significant amount of variability across all tissues, with the variation ranging from 60.63% to 90.19%. Additionally, the analysis revealed that all tissues generally had higher phenolic compound content in "ON" year trees compared to "OFF" year trees.

3.4.4. Mann–Whitney U test results

Mann–Whitney U, a nonparametric test, was used to determine the statistically significant differences in phenolics according to the explant source in the "ON" and "OFF" years. The results indicated that in 2015 data, catechin (at the 1% significance level) in shoots and quercetin (at the 10% significance level) in leaves differed statistically between "ON" and "OFF" year samples, with higher levels observed in the "ON" years compared to the "OFF" years (Table 6).

In 2016, the only difference observed was in the concentration of chlorogenic acid (at the 1% significance level) in leaves, which was higher in the "ON" years (Table 6).

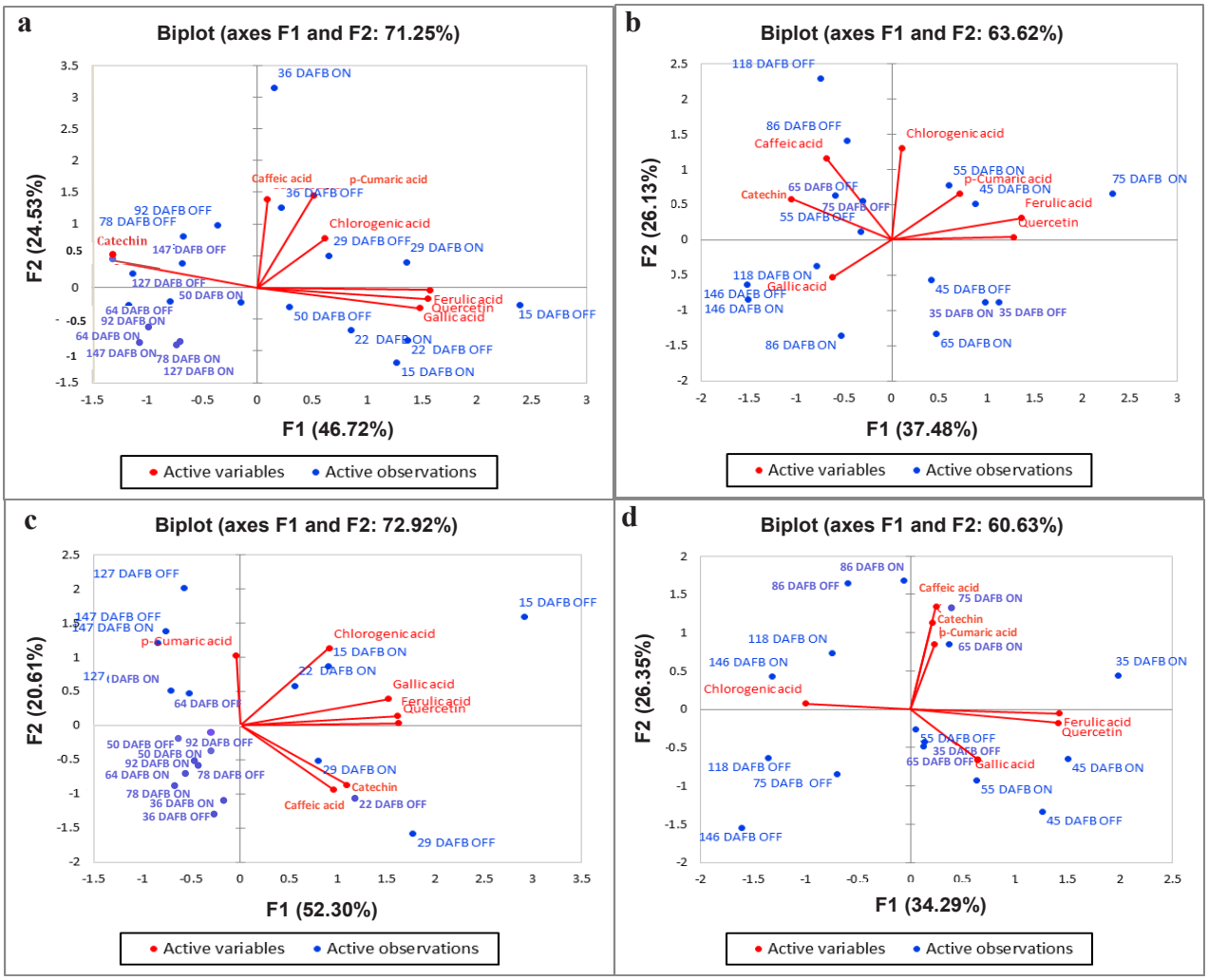

Figure 11. Biplot graph (scores and loading plots) obtained from principal component analysis, a) leaf, 2016, b) leaf, 2015, c) shoot, 2016, d) shoot, 2015.

4. Discussion

Pistachio nuts are rich in polyphenols, including anthocyanins, flavonols, proanthocyanidins, and isoflavones, which possess strong antioxidant properties and may offer protection against certain human diseases. The heavy crop load caused by alternate bearing cycles is believed to affect pistachio trees, leading to flower bud abscission between June and July (Goldschmidt and Golomb, 1982). Additionally, studies suggest that the flower bud abscission process in fruit trees, particularly in pistachios, is related to levels of endogenous biochemical compounds and the involvement of endogenous hormones during the stages of flower bud formation (Baktir et al., 2004; Mirsoleimani et al., 2018). Previous studies have failed to provide conclusive evidence regarding the regulatory role of phenolic compounds in the alternate bearing process. However, a substantial body of research suggests that phenolic acids and other phenols play a crucial role in influencing growth, morphogenesis, and metabolic activity in both in vivo and in vitro systems. It is postulated that a critical threshold level of changes in phenolic compounds may be necessary to trigger alternate bearing in fruit trees. The present study reports the differences in the properties of phenolic compounds in the shoot, leaves, panicle, and nuts of pistachio from "Uzun" variety. Our results also indicate a pattern of changes in the phenolic compound content in different parts, which is related to the alternate bearing cycle during both flower bud abscission and kernel development in "Uzun" pistachio trees. Studies have reported that pistachio fruits contain a rich phenolic content (Tokuşoğlu et al., 2005; Abidi and Akrimi, 2022; Moreno-Rojas et al., 2022). The findings of this study are largely consistent with these reports. The variation pattern of caffeic acid also demonstrated

Table 6. Results of the Mann–Whitney U test.

Year	Explant	Statistics	CA	GA	p-CA	ChA	CT	FA	QN
2015	Shoot	Mann–Whitney U	20	22.5	30	27	0	18	18
		Wilcoxon W	56	58.5	66	63	36	54	54
		Z	–1.26	–0.998	–0.21	–0.525	–3.361	–1.47	–1.47
		Asymp. Sig. (2-tailed)	0.208	0.318	0.834	0.6	0.001	0.141	0.141
		Exact Sig. [2*(1-tailed Sig.)]	0.234*	0.328*	0.878*	0.645*	0.000*	0.161*	0.161*
	Leaf	Mann–Whitney U	19	21	26	19.5	22	30	15
		Wilcoxon W	55	57	62	55.5	58	66	51
		Z	–1.365	–1.155	–0.63	–1.314	–1.05	–0.21	–1.785
		Asymp. Sig. (2-tailed)	0.172	0.248	0.529	0.189	0.294	0.834	0.074
		Exact Sig. [2*(1-tailed Sig.)]	0.195*	0.279*	0.574*	0.195*	0.328*	0.878*	0.083*
2016	Shoot	Mann–Whitney U	43.5	36	47	32	42	41	49
		Wilcoxon W	98.5	91	102	87	97	96	104
		Z	–0.492	–1.058	–0.227	–1.362	–0.605	–0.68	–0.076
		Asymp. Sig. (2-tailed)	0.623	0.29	0.82	0.173	0.545	0.496	0.94
		Exact Sig. [2*(1-tailed Sig.)]	0.631*	0.315*	0.853*	0.190*	0.579*	0.529*	0.971*
	Leaf	Mann–Whitney U	39	36	34	12	47	41	45
		Wilcoxon W	94	91	89	67	102	96	100
		Z	–0.832	–1.058	–1.21	–2.873	–0.227	–0.68	–0.378
		Asymp. Sig. (2-tailed)	0.406	0.29	0.226	0.004	0.821	0.496	0.705
		Exact Sig. [2*(1-tailed Sig.)]	0.436*	0.315*	0.247*	0.003*	0.853*	0.529*	0.739*

*Not corrected for ties. Caffeic acid (CA), gallic acid (GA), p-cumaric acid (p-CA), chlorogenic acid (ChA), catechin (CT), ferulic acid (FA), quercetin (QN).

that this phenolic compound may be associated with alternate bearing. The decreased concentration of caffeic acid in "OFF" trees up to 36 DAFB may indicate a lower level of metabolic activity compared to "ON" trees. This reduced metabolic activity could account for the observed decline in nut production during the "OFF" years. Moreover, caffeic acid possesses antioxidant properties and can scavenge reactive oxygen species (ROS) that cause cellular damage during plant senescence (Jajic et al., 2015). Hence, the lower concentration of caffeic acid in "OFF" trees may increase their vulnerability to ROS damage, which could further contribute to their reduced nut production. On the contrary, the significant increase in caffeic acid concentration in "OFF" trees during the

second phase of nut development implies that caffeic acid may play a crucial role in nut development. During this crucial period, an increased amount of caffeic acid may be essential for nut development. The reduced concentration of caffeic acid in "OFF" trees may contribute to their decreased nut production in alternate years. Conversely, the high caffeic acid content during the bud abscission period in nuts suggests that caffeic acid may play a crucial role in fruit development. It is possible that caffeic acid is essential for the fruits during this particular period and is transferred from the leaves and shoots to the developing nuts. Pichersky and Gang (2000) indicated that caffeic acid O-methyltransferase (COMT) enzyme methylates caffeic acid, leading to the formation of ferulic acid. Ferulic acid, in turn, is involved in the biosynthesis of lignin. This information supports the theory that the accumulation of caffeic acid in nuts during the nut development phase may be related to its role in lignin biosynthesis and cell wall strengthening.

Malik et al. (2015) observed that in the bud tissue of certain citrus species, the concentration of chlorogenic acid and naringenin increases when vegetative buds begin to sprout. Lavee et al. (1986) also demonstrated that the content of chlorogenic acid in olive leaves fluctuates in accordance with the alternate bearing cycle.

Chlorogenic acid can affect the activity of enzymes involved in photosynthesis and respiration, which are critical processes for the production and storage of energy in plants (Mersie and Singh, 1993; Song et al., 2022). Additionally, chlorogenic acid has been shown to regulate the expression of genes involved in the biosynthesis of plant hormones, which can affect the timing and intensity of fruiting (Lavee et al., 1986, 1993). On the other hand, the role of chlorogenic acid as an authentic intermediate in the lignin biosynthetic pathway is well-established. However, the mechanisms by which the chlorogenic acid pool is directed towards the production of lignin monomers in response to developmental or environmental signals remain unclear (e Silva et al., 2019). The low concentration of chlorogenic acid in pistachio organs during the "ON" year, compared to the "OFF" year, during nut development, may be associated with converting to lignin as an intermediate in the lignin biosynthetic pathway. However, the exact mechanisms by which chlorogenic acid affects alternate bearing in fruit trees are still not well understood and further research is needed to fully elucidate its role. Additionally, the concentration of gallic acid exhibited a decline in both leaves and shoots during "ON" and "OFF" years throughout the growing season. Additionally, there was a reduction in gallic acid concentration in nuts throughout the growing season. During the "OFF" year, the tree may accumulate more nutrients and energy for the following "ON" year, resulting in higher levels of phenolic compounds, such as gallic acid. Conversely, during the "ON" year, the tree may allocate more resources to fruit production, leading to a decrease in the concentration of gallic acid. The impact of p-coumaric acid on alternate bearing can be attributed to its concentration changes during specific periods. In the "ON" year, p-coumaric acid concentration increased in early May, while in the "OFF" year, it showed an increase during the bud abscission period. Interestingly, there was also an increase in p-coumaric acid concentration during the bud abscission period in both peduncles and nuts. Despite the overall increase in p-coumaric acid concentration in the nuts, a distinct peak was observed during a specific period. During this period, there was an increase in p-coumaric acid concentration in the "ON" year leaves and shoots, while a decrease was observed in the "OFF" year leaves and shoots. These findings suggest that the concentration of p-coumaric acid during critical periods may play a role in regulating alternate bearing, with variations observed between "ON" and "OFF" years and across different plant parts. p-coumaric acid is also a precursor of lignins (Goleniowski et al., 2013), which may explain its high concentration during kernel development.

Mirsoleimani et al. (2018) reported opposite changes in chlorogenic acid content in mandarin leaves between nonbearing and bearing trees, suggesting that the presence or absence of fruits may affect the concentration of chlorogenic acid in the leaves. Similarly, the concentration of chlorogenic acid was significantly different in the "ON" and "OFF" year leaves during the bud abscission period.

The fluctuations in catechin concentration and its different concentration in the "ON" and "OFF" years may be associated with the alternate bearing pattern of pistachio trees and the development of the nuts. Catechin functions as a vital antioxidant, protecting the plant from various biotic and abiotic stresses, particularly oxidative stress. During high-yield years, the plant may need a higher amount of catechin to protect the growing nuts from environmental stressors. Consequently, the concentration of catechin in the leaves gradually increases throughout the season. Conversely, in low-yield years, the plant may not require a high level of catechin, resulting in a decrease in the concentration of catechin in the leaves by the end of the season. However, there is a sudden increase in catechin concentration approximately 120 days after full bloom in low-yield years, which could be linked to nut development. This rise in catechin concentration may be due to the heightened demand for antioxidants as the nuts mature and approach harvest time. Rani et al. (2011) reported that catechin may be involved in Arabidopsis thaliana development processes such as the length of primary and lateral roots, the number of lateral roots, the fresh

and dry masses of shoots and roots, leaf area, the water potential of leaf and root tissues, the number of vascular bundles in the inflorescence, and leaf thickness. The concentration of ferulic acid in leaves and shoots showed a declining trend during the growing season. However, compared to other phenolic compounds, its concentration was higher in "ON" year trees than "OFF" year trees, with a noTable increase in late July. Mathew and Abraham (2004) highlighted that the dehydrodimers of ferulic acid are crucial structural components of plant cell walls, promoting rigidity and strength. Similarly, Pichersky and Gang (2000) pointed out the significant role of ferulic acid in lignin biosynthesis. The accumulation of ferulic acid concentration in peduncles and then in fruits during the early season, followed by its gradual decrease during nut development, could be attributed to its utilization in lignin formation. Although the overall quercetin concentration declined, fluctuations were observed between the "ON" and "OFF" annual trees. While quercetin concentration decreased during the growing season, it increased in June. Quercetin has been reported to be involved in the defence against insects, fungi, nematodes, and weeds. It also protects the plants from UV damage and stress conditions caused by pigment accumulation or lignification processes (Mierziak et al., 2014). The variations observed in quercetin concentration throughout the season may be attributed to its role in plant physiology. Our study demonstrated that the development of fruit in "Uzun" pistachio trees was influenced by the concentration of catechin in the leaves. Conversely, the concentration of ferulic acid, quercetin, caffeic acid, and gallic acid in the leaves, shoots, and nuts of "Uzun" pistachio trees were affected by the alternate bearing cycle, particularly during flower bud abscission and kernel development. In agreement with these Lavee et al. (1986), Tomaino et al. (2010) and, Mirsoleimani et al. (2018), it has been shown that phenolic compound contents of pistachio, mandarin, and olive fluctuate in correspondence with the alternate bearing cycle, respectively. Consequently, different trends in the changes of phenolic compounds in response to physiological processes have also been observed in other studies (Arcan and Yemencioğlu, 2009; Bujdosó et al., 2014; Erşan et al., 2016; Celik et al., 2017; Bodoira et al., 2019). The minor differences in the concentration of phenolic acid compounds in plants depend on numeorus factors, including environmental stress and growth conditions. In summary, the results suggest that the process of nut lignification alters the distribution of phenolic compounds within various organs, where these compounds serve as pivotal precursors of lignin synthesis. As a result, our investigation is motivated by the imperative of exploring the intricate correlations between these alterations and their potential implications for both flower bud formation and the developmental stages of the kernel. Although limited research has focused on phenolic compounds and their involvement in the physiological pathways leading to bud abscission in trees, the findings of this study may pave the way for future investigations aimed at revealing the exact mechanism of alternate bearing.

5. Conclusion

This study aimed to address a crucial and complex issue in pistachio cultivation, commonly referred to as alternate bearing. Given the pivotal role of secondary metabolites, particularly phenolic compounds, in plant physiology, we investigated the changes in these compounds during flower bud abscission in 'Uzun' pistachio trees, under both bearing and nonbearing conditions.

The activity of individual phenolic compounds has been shown to significantly affect flower bud abscission and kernel development. Therefore, determining the changes in the concentration of phenolic compounds in different organs of both "ON" and "OFF" year trees during the bearing period may contribute to a better understanding of the physiological mechanisms underlying alternate bearing in pistachio. Furthermore, the potential health benefits of phenolic compounds should not be overlooked. Our results suggest that the process of nut lignification alters the distribution of phenolic compounds in the organs, which serve as precursors of lignin. However, the trend in concentration changes differs between both "ON" and "OFF" year trees. Although limited research has focused on phenolic compounds and their role in the physiological pathways leading to bud abscission in trees, the findings of this study may pave the way for future investigations aimed at revealing the exact mechanism of alternate bearing.

Acknowledgments

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