Quantitative variation of phenolic compounds in different tissues of pistachio (*Pistacia vera* L. cv. UZUN) related to alternate bearing

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Abstract: Alternate bearing is a common challenge in horticultural plants, leading to irregular yield in successive years. However, the potential role of phenolic compounds in regulating this phenomenon is not well understood. This study aimed to investigate

the possible role of phenolic compounds on alternate bearing in pistachio trees by analyzing 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 analyzed using high-performance liquid chromatography at 10-day intervals. Significant variations were observed in the levels of some phenolic compounds in "ON" and "OFF" years, suggesting the possible role of phenolic 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 economically most important 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; Gundesli et al., 2020a). Turkey is one of the origins of pistachio has important genetic sources and is the center of the generation and evolution of new pistachio varieties (Gundesli et al., 2019). However, alternate bearing is a significant problem that affects pistachio production,

resulting in irregular fruit yields across 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 a lack of consistent yields. Despite many efforts to explain this unusual phenomenon, the underlying mechanisms remain unknown. As a result, alternate bearing negatively impacts consumers, producers, and the economy of the country (Hormaza et al., 2007; Okay et al., 2011; Goldschmidt, 2013; Gundesli, 2020; Khezri et al., 2020).

Some studies suggest that alternate bearing in pistachio trees is influenced not only by genetic factors but also by environmental and physiological factors, cultural management practices (Esmaeilpour and Khezri, 2006), tree nutrient balance (Baninasab and Rahemi, 2006; Baninasab et al., 2007; Gunes 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., 2020b; 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) and play a crucial role in the growth and reproduction of plants, especially in the defence mechanisms (Bravo, 1998). Phenolic compounds also influence the taste of horticultural crops, including pistachios, which are a good source of various polyphenolic compounds (Tokusoglu et al., 2005; Bodoira et al., 2019). Tomaino et al. (2010) identified 17 phenolic and polyphenolic compounds in pistachio nut and skin extracts, while Ersan 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; Martinez 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 Yemenicioglu, 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 are important in the control of 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 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 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 Dr. Ahmet Munir's experimental region at the Gaziantep Pistachio Research Center. Known for its exceptional aroma and flavor, the "Uzun" cultivar holds great value in Turkey. It is characterized by a sturdy, semi-upright tree structure and is classified as a mid-flowering cultivar.

In 2013, a total of 40 trees were chosen 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 - 2016, samples of leaves, shoots, peduncles, and fruits were collected in 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. In addition, the climate data of the region, obtained from the General Directorate of Meteorology, were analysed to allow 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 in cold chain condition thereafter were dried in lyophilizer 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 hour. The resulting extract was then adjusted to a final volume of 10 ml and centrifuged at 4500 rpm for 15 minutes. The supernatant was filtered through a 0.2 µm filter and subsequently analyzed using HPLC.

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) with a DAD (photodiode Array

Detector) was used to detect and quantify seven specific phenolic compounds including 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 detection of the compounds took place in the wavelength range of 280 nm to 360 nm. Elution was performed with a non-linear gradient of a solvent mixture of 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 reached 13% in 15 minutes, followed by an increase to 15% in 5 minutes, then to 30% in another 5 minutes, which was maintained for 3 minutes. There was then a gradual increase to 45% in 4 minutes, which was maintained for 3 minutes, a further increase to 90% in 5 minutes and the maintenance of this level for 5 minutes before returning to baseline conditions within 5 minutes. 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), the Mann Whitney u-test, the t-test and the logit regression were used. Mann-Whitney u test was used to investigate whether the phenolic levels in different organs have a statistical significance in on and off trees. This nonparametric method was chosen because the number of observations was low based on organs and the distribution of data could not meet the normality condition. SPSS program, XLSTAT,

STATA softwares 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, our primary objective 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. Moreover, the study aimed 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 08.04.2015 and 10.04.2016. Despite the same temperature changes in both years, the higher humidity levels in 2015 may have led to an 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 (Table 2, 3 and 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 "ON" year than "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 in the two years of the study. In contrast, during the "OFF" year, the range of caffeic acid content was between 17.70 µg/g and 43.57 µg/g in 2015 and between 17.84 µg/g and 30.62 µg/g in 2016. The caffeic acid contents of Leaves in "OFF" trees were significantly lower than "ON" trees until 36 DAFB. Thereafter, the caffeic acid concentration increased sharply in "OFF" trees while increased in "ON" trees revealing 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 exhibited variation between 20.41 µg/g and 45.32 µg/g across the two years of the study (Figure 3). The variation of gallic acid concentration was recorded between 1.97 - 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 year 2016 in both "ON" and "OFF" years which coincides with the first phase of bud abscission. However, the amount of gallic acid in "ON" years was significantly lower than "OFF" years. Quercetin was the second major phenolic compound in the leaves and its concentration was ranged between 49.82 - 113.45 µg/g in studied samples. The amount of quercetin in "ON" years was slightly higher than in "OFF" years especially in year 2015. In the "ON" years, the amount of quercetin was slightly higher than in the "OFF" years, especially in 2015. During 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 non-bearing 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 non-bearing and its content ranged from 10.61 - 19.71 µg/g in all tested samples (Figure 3).

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

Similar to leaves, ferulic acid was the predominant phenolic compound in the shoots. The amount of ferulic acid varied between 28.30 - 190.53 µg/g and 17.85 - 293.20 µg/g in "ON" and "OFF" years, respectively. The amount of ferulic acid was 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 in different bearing years and in studied years. Its concentration varied between 10.47 - 23.34 µg/g and 5.08 - 12.30 µg/ in 2015 and 2016, respectively in "ON" year and between 1.19 - 24.23 µg/g and between 5.78 - 14.95 µg/g in 2015 and 2016, respectively in "OFF" year. The amount of chlorogenic acid varied between 16.31-70.99 µg/g among 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 "OFF" years than

"ON" years. However, its concentration decreased gradually in "OFF" years throughout the season, except for a sudden increase in mid-June. The amount of gallic acid reached a minimum level during June, which coincided with a period of intensive bud abscission. The shoot samples under study showed a quercetin concentration range of 23.07 - 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 and 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, with a range of 2.16 - 21.96 µg/g. The concentration of catechin, the second major phenolic compound in shoots, varied from 29.16 to 227.89 µg/g in the studied samples. In particular, the concentration of catechin was significantly higher in 2015 compared to 2016, and the changes in "ON" and "OFF" years were also distinct in both years. The amount of p-coumaric acid varied between 9.52 - 14.05 µg/g in among all samples. No differences were detected in p- coumaric acid concentration between "ON" and "OFF" years and similar changes were recorded in most periods (Figures 4 and 5).

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 during late June. In contrast, the concentration of ferulic acid in nuts was high at the beginning of the season and then showed a sharp decline during nut development to reach its minimum level. The concentration of

caffeic acid ranged between 2.51 - 14.00 µg/g and 2.12 - 8.22 µg/g in peduncle and nuts, respectively in 2016 (Figure 7). Despite low concentration at the beginning of the season, the concentration of caffeic acid increased in peduncles during fruit development while the amount of caffeic acid in the nuts was high at beginning of the season and then decreased and remained at a constant state in low level till May in 2016. The amount of chlorogenic acid varied between 15.25 - 126.66 µg/g and 16.33 - 46.26 µg/g in peduncle and nuts, respectively in 2015 (Figure 8). Chlorogenic acid concentration in peduncles was high during May and decreased slightly in June, followed by an increase near the harvest time. On the other hand, in nuts, the concentration of chlorogenic acid was high during 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 between 4.74 - 14.46 µg/g and 1.37 - 57.97 µg/g in peduncle and nuts, respectively in 2015. The high concentration of gallic acid decreased until about 65 DAFB, the time of buds abscission and then remained at the same level in 2015. Likewise, in nuts, the concentration of gallic acid was initially high at the beginning of full bloom but gradually decreased as the season progressed. It reached a minimum near harvest time (Figure 8). The amount of quercetin varied between 18.13 - 41.61 µg/g and 0.32 - 36.76 µg/g in peduncles and nuts, respectively in 2015. The concentration of quercetin in peduncles exhibited a remarkable decline in May, in contrast to its high concentration 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 the harvest (Figure 9). The amount of catechin varied between 65.97 - 323.11 µg/g and 45.42 - 94.45 µg/g in peduncle and nuts, respectively in 2015. Catechin concentration of

peduncle was low approximately 35 DAFB and showed a peak 55 DAFB following a decrease. After an initial slight increase, a downward trend was observed until harvest time. Similarly, catechin concentration of nuts was high from mid-May until mid-June. However, its amount increased during the seed filling period followed by a decrease 65 DAFB (Figure 9). The concentration of p-coumaric acid ranged between 11.06 - 13.16 µg/g and 10.58 - 12.66 µg/g in peduncle and nuts, respectively in 2015. In peduncles, the concentration of p-coumaric acid increased gradually throughout the season. However, in nuts, the amount of p-coumaric acid was at a high level from 35 to 55 DAFB, then decreased, and increased again near the harvest time (Figure 9).

3.4. Statistical analyses results

3.4.1. t-test of Results

The 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 (Table 2 and 3). The results given in Tables 2 and 3 are the mean concentration of triplicate analysis. When "ON" and "OFF" are compared, caffeic, gallic, Chlorogenic, Ferulic and quercetin were observed lower in "ON" years than "OFF" years. p-pumaric and catechin were higher in "ON" years. The largest fluctuation was observed in ferulic acid in both "ON" and "OFF" years. The fluctuation of caffeic, gallic, and ferulic acid and quercetin is lower in "ON" years based on sampling time. Caffeic acid and catechin levels were significantly different in "ON" and "OFF" years (considering all explant sources together) (Table 2 and Table 3). Caffeic was low versus high catechin concentration in "ON" years (Table 3). The only difference between the two studied years in the results of the t-test was catechin which was unsense in 2016.

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

Regression is estimated for all variables separately in Tables 4 and 5. Caffeic, gallic, chlorogenic, ferulic acids, and quercetin significantly and negatively affected by "ON" year trees. Tables 4 and 5 shows the correlation coefficients between phenolic compounds and the alternate bearing tested. Some features have been found to be strongly interrelated. Negative and positive correlations at a significantly high level were found 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. 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 (CAMO Software AS, 1998; Kara, 2009). Here, PCA were 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 to regulate bud abscission. In 2015, PC1 and PC2 explained 46.59% and 22.07% of the total variance in nut samples. 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. Again, the first days 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 seems that the first sampling days (14 - 22 DAFB) differ from the others. Especially day 29 has the most difference. Besides, it was observed that the phenolic levels were higher in the first days and decreased especially during the fruit filling period (Figure 10).

In 2015, while PC1 explained 47.09% of the total variance, PC2 explained 35.99% in peduncle samples. These two components may explain 83.08% of the total variance. Although there is no serious grouping here, it is seen that caffeic acid has increased in recent days (Figure 10). In 2016, PC1 could explain 56.10% of the total variance, while PC2 could explain 23.90%. These two components can explain 80.00% of the total variance. In general, it is seen that the first days form a group and again the phenolic levels, in general, are higher these days. The last days can be evaluated in two groups, 36-50-78 is one group and 64-92-127 is the other group (Figure 10).

In 2015, PC1 and PC2 explained 37.48% and 26.13% of the total variance in leaf samples, respectively. The variance that the two factors can explain was 63.62% of the total variance. Caffeic acid and Catechin increased in the "OFF" year, while p-coumaric, ferulic, and quercetin were higher in the "ON" year. Apart from this, there is not a very clear grouping, but it has been observed that there are some differences from each other in the same period. When considered independently from bearing status, it is seen that the first days and the last days are separated (Figure 11). In 2016, it is seen that PC1 and PC2 explained 47.72% and 22.53% of the total variance, respectively. The variance that the two factors can explain is 71.25% of the total variance. Many phenologies appear to be lower in "ON" years and the last days of sampling dates. Catechin is more in the "OFF" year (sampling date 78-92-12), as in the last days. When

considered independently from ON-OFF, it is seen that the first days and the last days are separated and the phenolic amount in the first days is higher (Figure 11).

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

Some important differences between phenolic compounds and alternate bearing were confirmed by PCA analysis. Overall, a significant amount of variability was found in the PCA results across all tissues. The overall variation covered a range from 60.63% to 90.19%. At the same time, it showed that all tissues analysed generally had higher phenolic compound content in "ON" year trees than "OFF" year trees.

3.4.4. Mann Whitney U test results

Mann Whitney U, a nonparametric test was used to understand the statistically significant difference in phenolics according to the explant source used in the "ON" and "OFF" years. According to the results of this test, in 2015 data, catechin (at 1% significance level) in shoots and quercetin in leaves (at 10% significance level) differed statistically in "ON" and "OFF" year samples and were higher in "ON" years compared to "OFF" years (Table 6).

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

4. Discussion

Pistachio nuts are rich in polyphenols, such as 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 flower bud formation stages (Baktir et al., 2004; Mirselomani et al., 2018). Previous studies have failed to provide conclusive evidence for the regulatory role of phenolic compounds in the alternate bearing process. However, a wealth 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 phenolic compound changes may be necessary to trigger alternate bearing in fruit trees. The present work reports the differences in phenolic compound properties shoot, leaves, panicle, and nuts of pistachio from "Uzun" variety. Our results also indicate the pattern of changes in different parts of phenolic compound contents that it is related to alternate bearing cycle during both flower bud abscission and kernel development in "Uzun" pistachio trees. Studies reported that pistachio fruits contain rich phenolic content (Tokusoglu et al., 2005; Abidi and Akrimi, 2022; Moreno-Rojas et al., 2022). The findings of this study are mostly in agreement with these reports. The variation pattern of caffeic acid also demonstrated 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 in comparison to "ON" trees. This reduced metabolic activity could be accountable for the decline in nut production during "OFF" years. Moreover, caffeic acid possesses antioxidant properties and can scavenge reactive oxygen species (ROS) that can 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 additionally contribute to their reduced nut production. On the contrary, the significant surge in caffeic acid concentration in "OFF" trees during the second phase of nut development implies that caffeic acid may play a crucial part in nut development. During this crucial period, an increased amount of caffeic acid may be essential for nut development, and the reduced concentration of caffeic acid in "OFF" trees may add to their reduced nut production in alternate years. On the other hand, the high caffeic acid content during 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 some citrus species, the concentration of chlorogenic acid and naringenin increases when vegetative buds begin to sprout. Lavee et al. (1986) have also shown 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 and 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 can be directed towards the production of lignin monomers in response to developmental or environmental signals remain unclear (e Silva et al., 2019). Low concentration of chlorogenic acid in pistachio organs in "ON" compared to OFF" year during nut development may associated with converting to lignin as its intermediate role in 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. The concentration of gallic acid exhibited a decline in both leaves and shoots of "ON" and "OFF" years over 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 "ON" year leaves and shoots, while a decrease was observed in "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 different plant parts. p-coumaric acid is also the precursors 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 in non-bearing and bearing trees, suggesting that the presence or absence of fruits may affect the concentration of chlorogenic acid in leaves. Similarly, the concentration of chlorogenic acid was considerably different in "ON" and "OFF" year leaves during bud abscission period.

The fluctuations in catechin concentration and its different concentration in "ON" and "OFF" years may associate with the alternate bearing pattern of the pistachio trees and the development of the nuts. Catechin acts as a vital antioxidant that shields the plant against different biotic and abiotic stresses, particularly oxidative stress. During high-yield years, the plant may need a greater amount of catechin to safeguard the growing nuts against environmental stressors. As a result, the concentration of catechin in the leaves gradually increases throughout the season. Conversely, during low-yield years, the plant may not require a high level of catechin, causing the concentration of catechin in the leaves to decrease towards the end of the season. However, there is a sudden spike in catechin concentration roughly 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 involve in Arabidopsis thaliana development processes such as length of primary and lateral roots, number of lateral roots, 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) have highlighted that the dehydrodimers of ferulic acid are crucial structural components of plant cell walls that promote rigidity and strength. Similarly, Pichersky and Gang (2000) have pointed out the significant role of ferulic acid in lignin biosynthesis. The accumulation of ferulic acid concentration in the early season in peduncles and then fruits, followed by its gradual decrease during nut development, could be attributed to its utilization in lignin formation. Quercetin concentration declined overall, but fluctuations were opposite in the "ON" and "OFF" annual trees. Although 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 against UV damage and stress conditions caused by pigment accumulation or lignification processes (Mierziak, 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, specifically during flower bud abscission and kernel development. In agreement with these Lavee et al. (1986), Tomaino et al. (2010) and, Mirsoleimani et al. (2018) have shown that phenolic compound contents of pistachio, mandarin, and olive fluctuate in correspondence with the alternate bearing cycle, respectively. Consequently, a different trend in the changes of phenolic compounds in response to physiological processes has also been observed in some other studies (Arcan and Yemencioglu, 2009; Bujdosó et al., 2014; Ersan et al., 2016; Celik et al., 2017; Bodoira et al., 2019). The minor differences in concentration of phenolic acid compounds in the plants depend on so many factors including environmental stress and growth conditions. Briefly, 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 tackle a crucial and complex issue within pistachio cultivation, commonly referred to as Alternate Bearing. Given the pivotal role of secondary metabolites, particularly phenolic compounds, in plant physiology, we investigated changes in phenolic compounds during flower bud abscission in 'Uzun' pistachio trees,

encompassing both bearing and non-bearing conditions. The activity of individual phenolic compounds has been shown to have significant effects on flower bud abscission and kernel development. Thus, determining the changes in the concentration of phenolic compounds during the bearing period in different organs of both "ON" and "OFF" year trees may contribute to a better understanding of the physiological mechanisms underlying alternate bearing in pistachio. Furthermore, the potential health benefits of phenolic compounds cannot be overlooked. Our results suggest that the process of nut lignification alters the distribution of phenolic compounds in organs, which serve as precursors of lignin. On the other hand, the concentration change trend is different in both "ON" and "OFF" year trees. 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.

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Table 1. Sampling dates of biochemical analyses.

2015 Sampling dates		2016 Sampling dates	
-	-	15 DAFB	22.04.2016
-	-	22 DAFB	29.04.2016
35 DAFB	15.05.2015	29 DAFB	06.05.2016
45 DAFB	25.05.2015	36 DAFB	13.05.2016
55 DAFB	04.06.2015	50 DAFB	27.05.2016
65 DAFB	14.06.2015	64 DAFB	10.06.2016
75 DAFB	24.06.2015	78 DAFB	24.06.2016
86 DAFB	05.07.2015	92 DAFB	08.07.2016
118 DAFB	06.08.2015	127 DAFB	12.08.2016
146 DAFB	03.09.2015	147 DAFB	01.09.2016

*DAFB = Days After Full Bloom

*Full bloom date respectively in the study years: 10.04.2015, 08.04.2016

Table 2. t-Test for 2015 data

Phenolic	ON / OFF	Observations	Mean ± SD	t	prob
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 for 2016 data

Phenolic	ON / OFF	Observations	Mean ± SD	t	prob
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" x "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)
_ cons	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; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
caffeic acid (CA), gallic acid (GA), p-cumaric acid (p-CA), chlorogenic acid (ChA), catechin (CT), ferulic_acid (FA), quercetin (QN)

Table 5. Pooled logit regression analysis for the year 2016

R.E.F. "ON" x "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)
_ cons	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 fort statistics in parentheses; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
caffeic acid (CA), gallic acid (GA), p-cumaric acid (p-CA), chlorogenic acid (ChA), catechin (CT), ferulic_acid (FA), quercetin (QN)

Table 6. Mann Whitney U-Test Results

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.)]	.234*	.328*	.878*	.645*	.000*	.161*	.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.)]	.195*	.279*	.574*	.195*	.328*	.878*	.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.)]	.631*	.315*	.853*	.190*	.579*	.529*	.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.)]	.436*	.315*	.247*	.003*	.853*	.529*	.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)

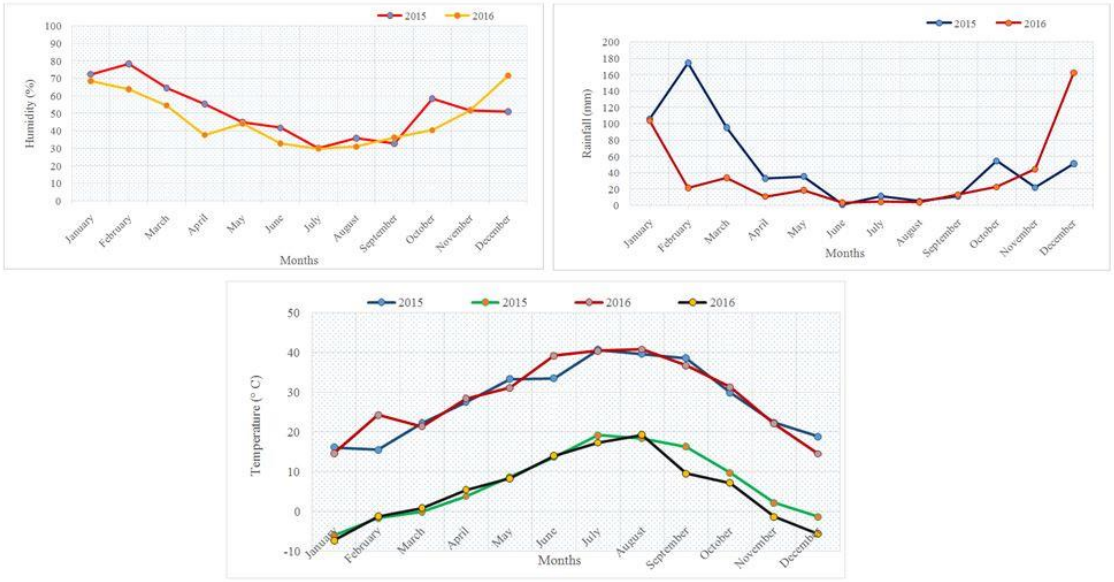


Figure 1. Metrological data of experiment field during season (2015 and 2016 years).

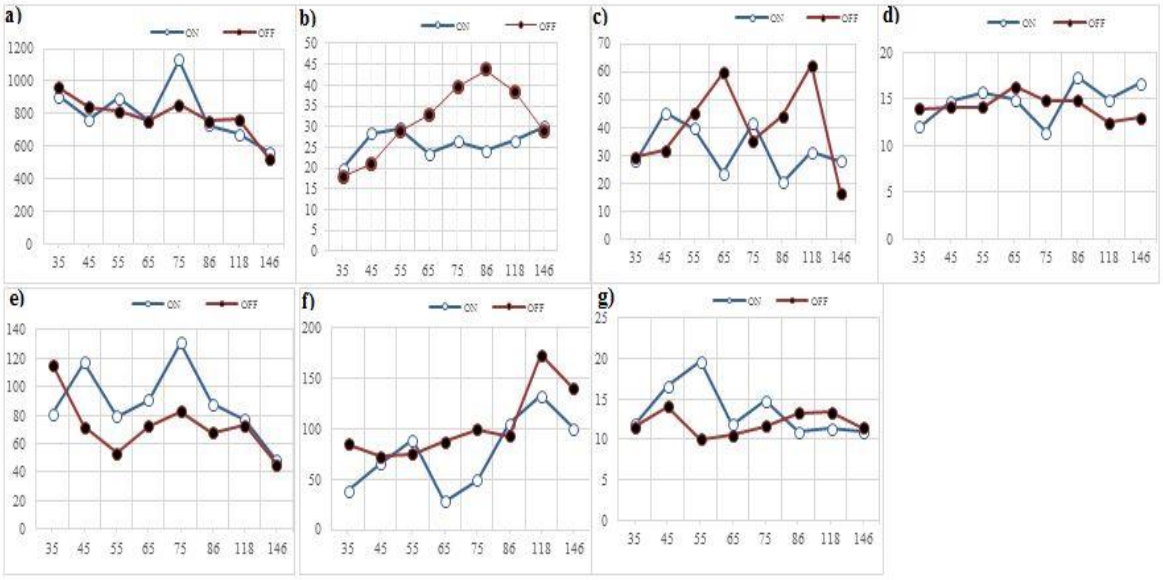


Figure 2. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in leaves of "OFF" and "ON" trees in year 2015, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

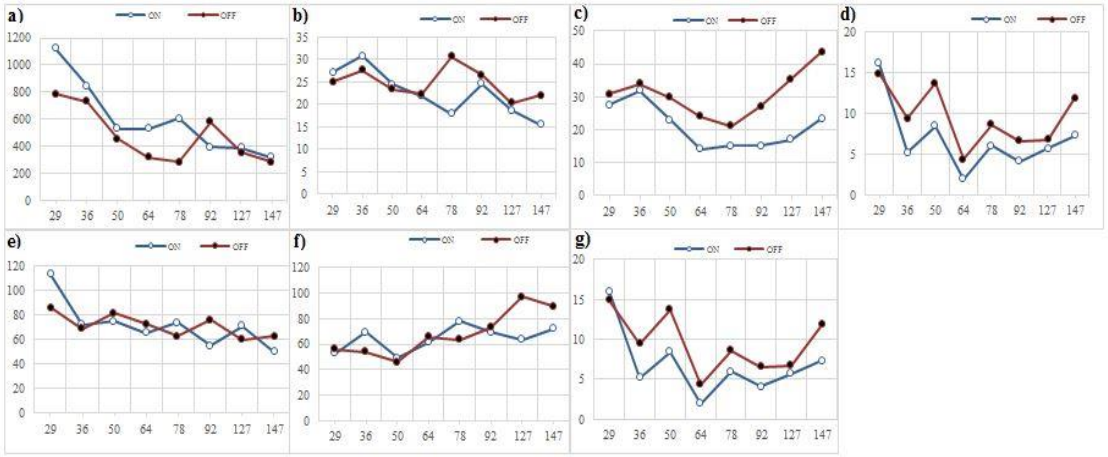


Figure 3. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in leaves of "OFF" and "ON" trees in year 2016, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

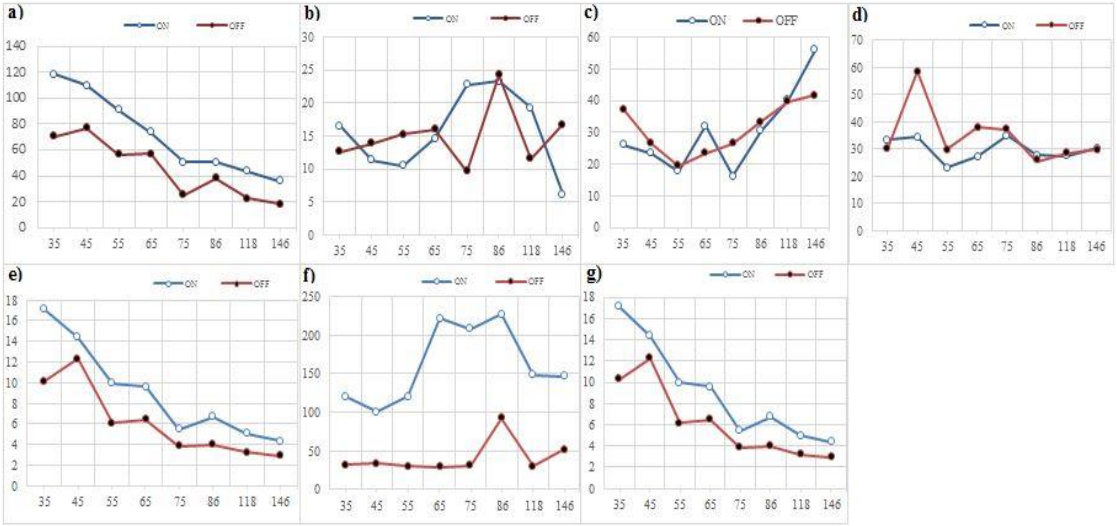


Figure 4. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in shoots of "OFF" and "ON" trees in year 2015, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

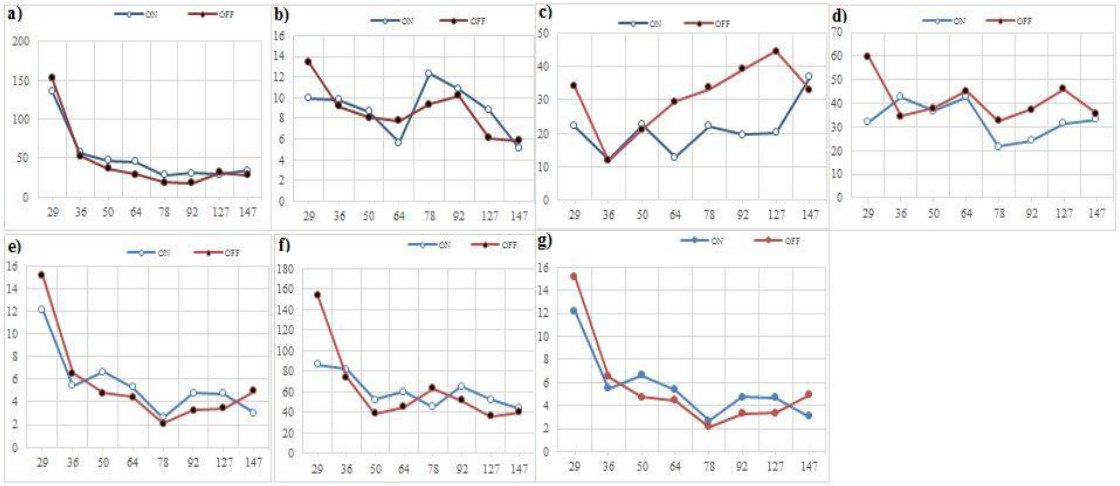


Figure 5. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in shoots of "OFF" and "ON" trees in year 2016, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

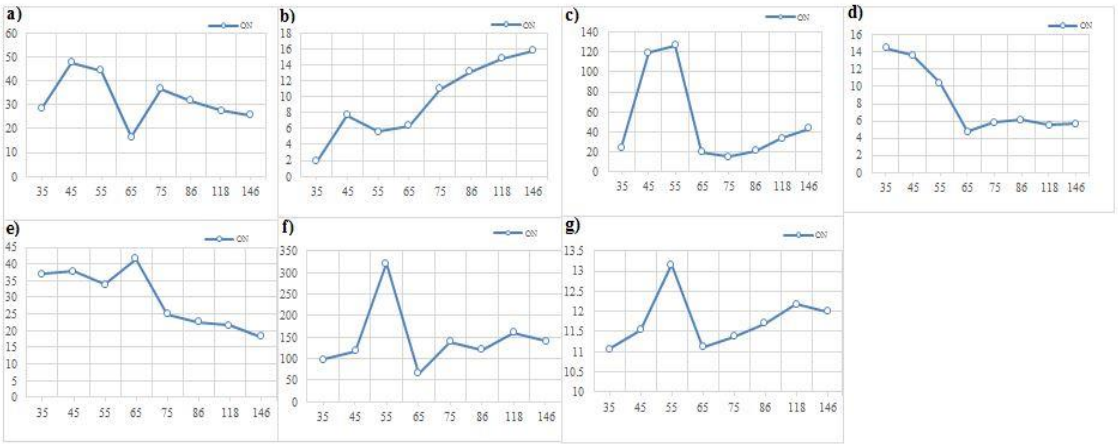


Figure 6. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in Peduncles "ON" trees in year 2015, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

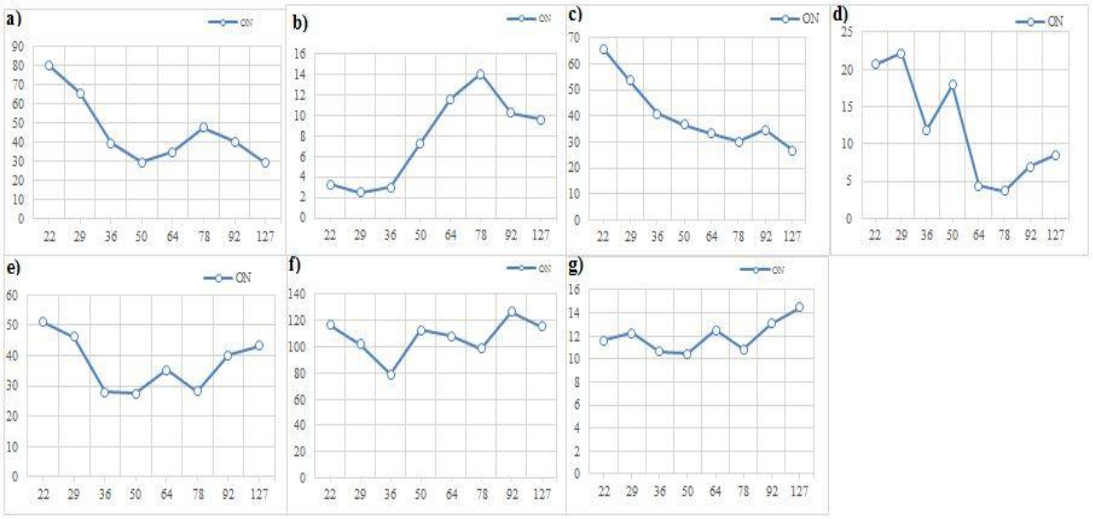


Figure 7. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in Peduncles of "ON" trees in year 2016, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

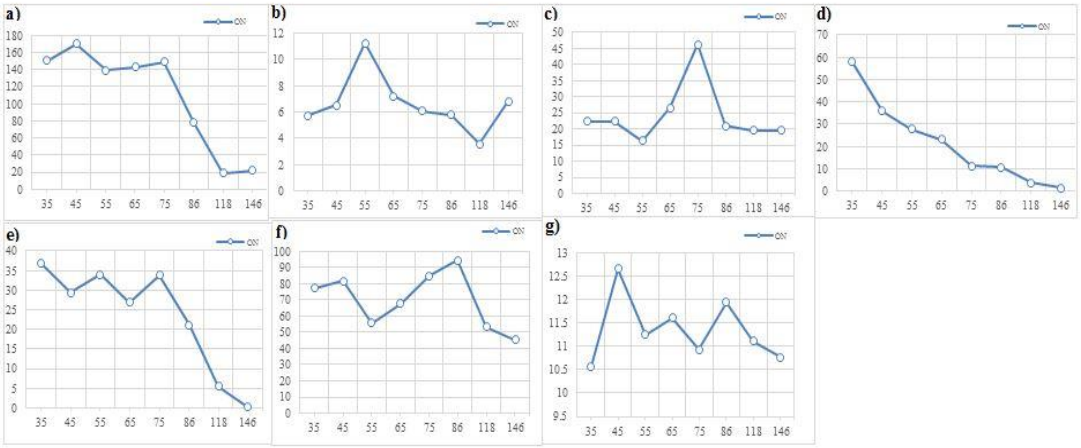


Figure 8. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in Nuts of "ON" trees in year 2015, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

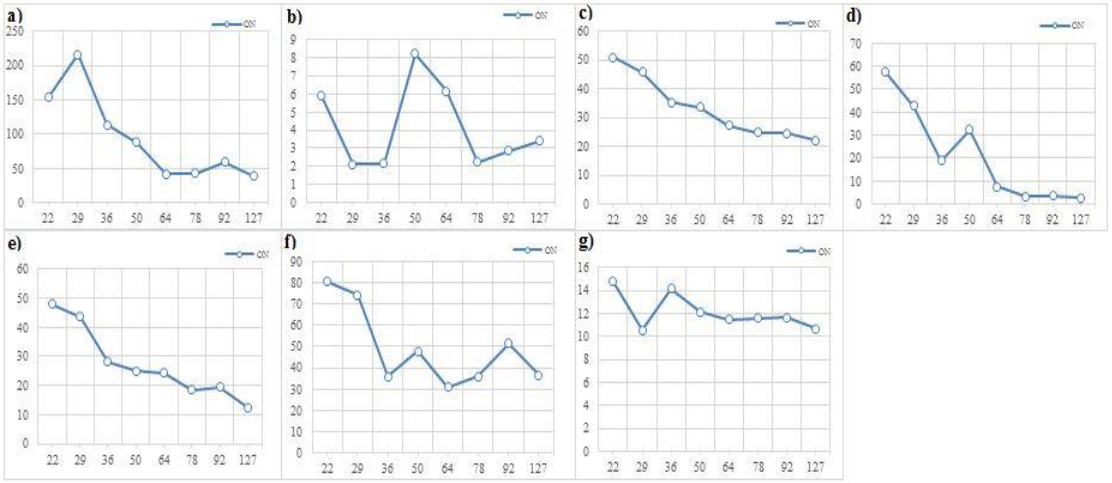


Figure 9. Variations of a)Ferulic acid, b)Caffeic acid, c)Chlorogenic acid, d)Gallic acid, e)Quercetin, f)Catechin, g) p-Cumaric acid in Nuts of "ON" trees in year 2016, y - axis: Date After Full Bloom; x - axis: Concentration in (µg/g)

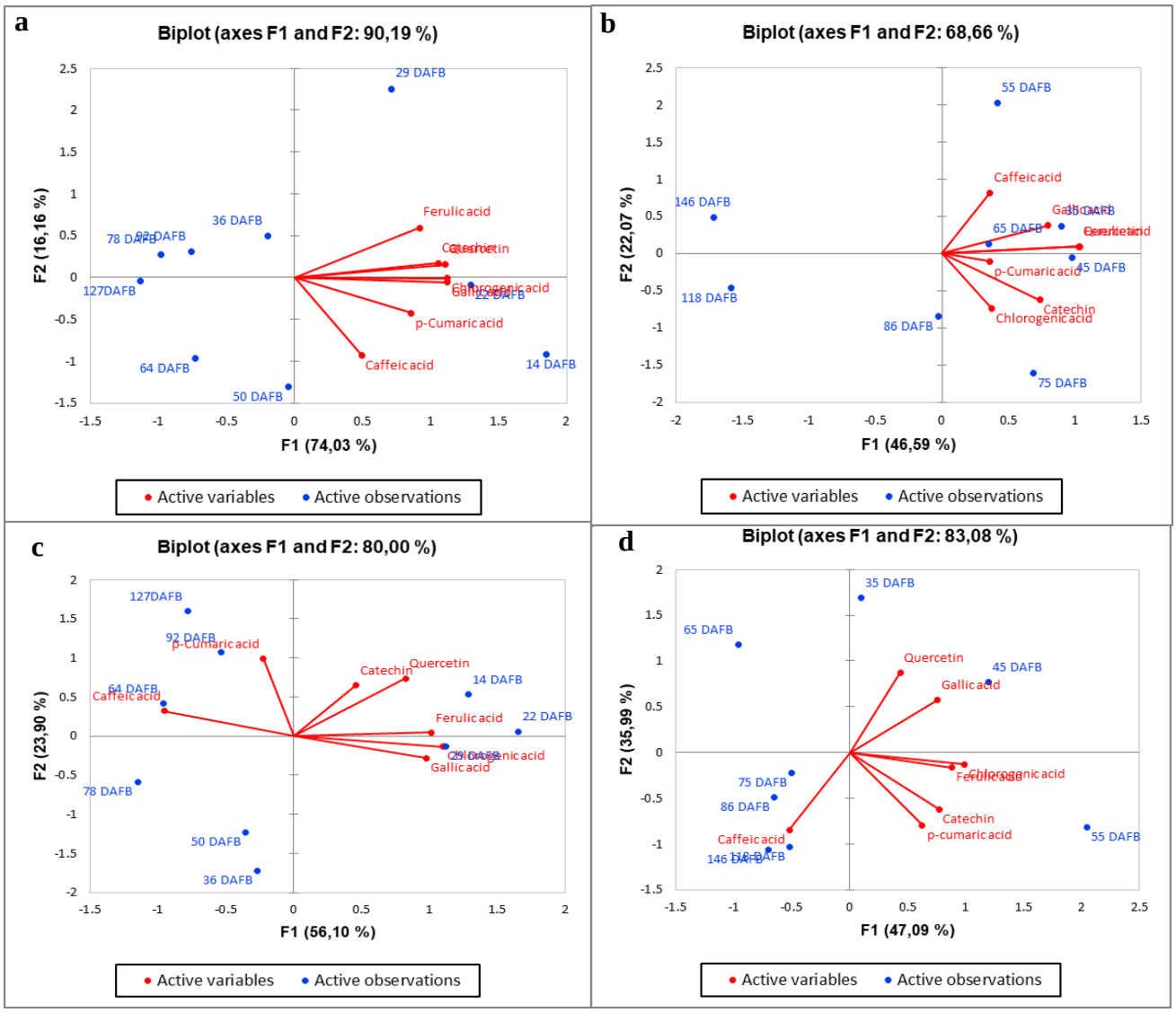
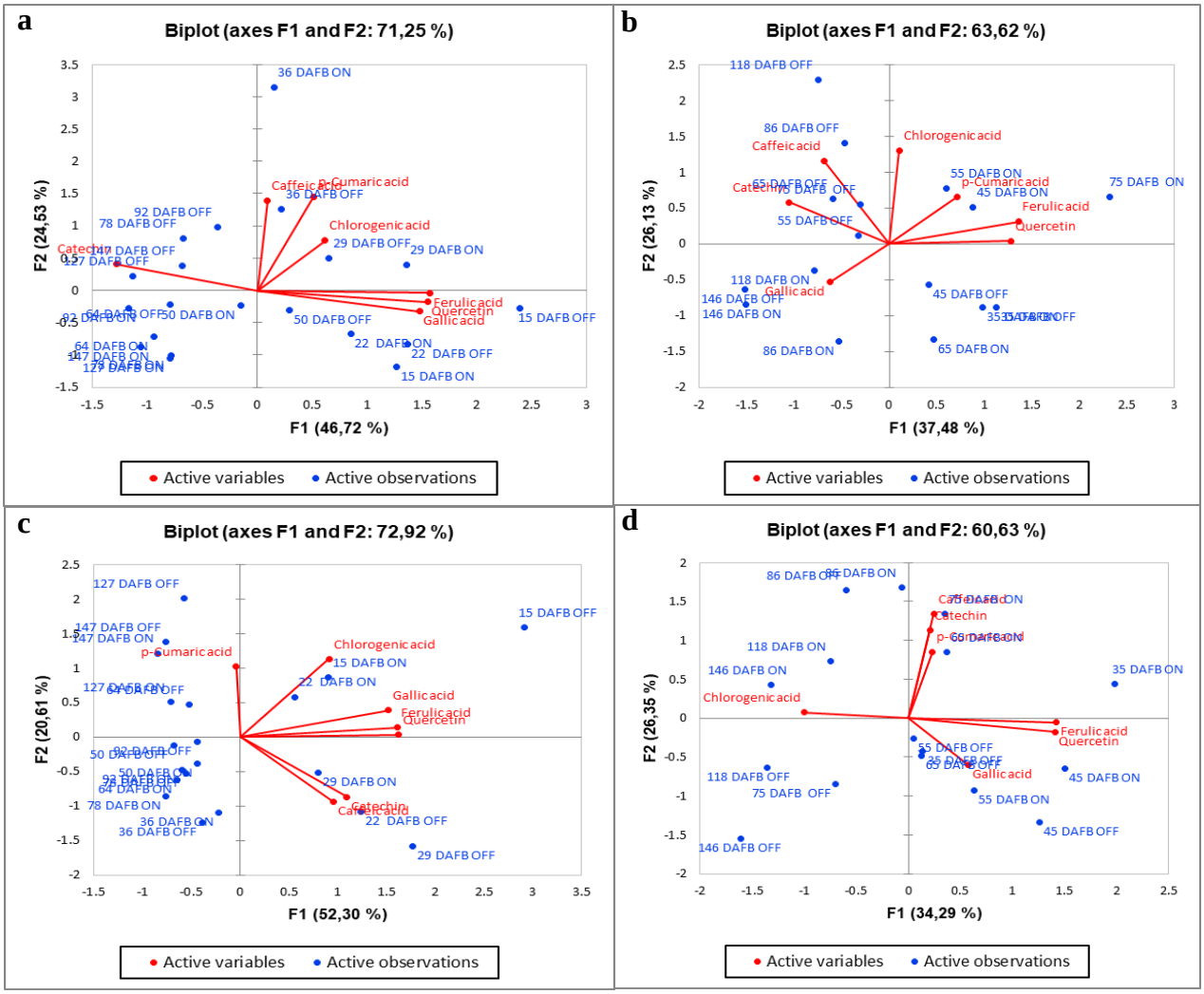


Figure 10. Biplot graph (scores and loading plots) obtained from Principal Component Analysis, A) Nut 2016 B) Nut 2015 C) Ped, 2016 D) Ped, 2015.



1 Figure 11. Biplot graph (scores and loading plots) obtained from Principal Component
2 Analysis, A) Leaf 2016 B) Leaf 2015 C) Shoot 2016 D) Shoot 2015.