CUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Mehmet Zafer DOĞU

EFFECIACY OF CLUSTER THINNING ON SEMIQUINONE RADICALS, PHYTOHORMONES, SUGAR UPTAKE AND PLANT NUTRIENTS IN DIFFERENT ORGANS OF PISTACHIO

DEPARTMENT OF HORTICULTURE

ADANA-2021

ABSTRACT

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Year: 2021, Page: 63
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Pistachios are known as “Green Gold” in the Middle East region, and provide important revenue for producers. Pistachio cultivation is done economically and has a big potential in Turkey. “Kirmizi” is one of the commercially grown varieties produced numerously in Turkey. Since pistachio is a type of nut that shows alternate bearing phenomena, and so production varies according to years and countries. The main goal of this thesis was to examine the effect of the flower cluster thinning application, on metabolic responses of pistachio (*Pistacia vera* L.cv. Kirmizi) depending on the time that has elapsed since making the flower cluster thinning. The first objective was to determine whether the above treatment alters the generation of semiquinone radicals in pistachio organs (fruits, leaves and shoots). The second objective was to verify whether it causes elevated generation signaling molecules, i.e. phytohormones such as abscisic acid (ABA), salicylic acid (SA) and indole-3-acetic acid (IAA) and changes in the sequence/period of their generation, and in the transduction of signals from organ to organ. Moreover, the third objective was to determine whether changes in the sugar levels occur after the flower cluster thinning. Additionally, it was aimed to determine the nutrient element concentrations in leaves, shoots and fruits after the flower cluster thinning application in the “Kırmızı” pistachio variety

Key Words: Pistachio, Cluster Thinning, Sugar, Mineral Nutrients

ÖZ

YÜKSEK LİSANS TEZİ

SALKIM SEYRELTMESININ ANTEPFISTIĞININ (*PISTACIA VERA* L.) FARKLI ORGANLARINDA SEMIKINON RADIKALLER, HORMONLAR, ŞEKER İÇERIKLERI VE BITKI BESIN ELEMENTLERI ÜZERINE ETKILERININ BELIRLENMESI

Mehmet Zafer DOĞU

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
BAHÇE BİTKİLERİ ANABİLİM DALI

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Türkiye'de "Yeşil Altın" olarak bilinen Antep fıstığı, özellikle Orta Doğu bölgesinde üreticiler için önemli bir gelir sağlıyor. Antep fıstığı, Türkiye'de ekonomik açıdan önemli bir sert kabuklu meyve ağacıdır. "Kırmızı" Türkiye'de en çok üretilen ticari çeşitlerden biridir. Antep fıstığı, periyodisite gösteren bir sert kabuklu meyve türü olduğu için üretim yıllara ve ülkelere göre değişiklik göstermektedir. Bu tezin temel amacı, çiçek salkımı seyreltmesi uygulamasının, çiçek salkımının seyreltmesinden derime kadar geçen süreye bağlı olarak fıstığın (*Pistacia vera* L.cv. Kırmızı) metabolik tepkileri üzerindeki etkisini incelemektir. Birinci amaç, seyreltme uygulamasının antepfıstığı organlarında (meyveler, yapraklar ve sürgünler) semikinon radikallerinin oluşumunu değiştirip değiştirmediğini belirlemektir. İkinci amaç, sinyal moleküllerine, yani absisik asit (ABA), salisilik asit (SA) ve indol-3-asetik asit (IAA) gibi fitohormonlara ve bunların üretim sırası/periyodundaki değişikliklere neden olup olmadığını doğrulamak ve sinyallerin organdan organa iletilmesindeki etkisini araştırmaktır. Ayrıca üçüncü amaç, çiçek salkımının seyreltmesinden sonra şeker seviyelerinde değişiklik olup olmadığını belirlenmesidir. Bunlara ek olarak Türkiye ve özellikle Güneydoğu Anadolu bölgesinde mutlak periyodisite gösteren en önemli çeşitlerden biri olan "Kırmızı" fıstık çeşidinde çiçek salkımı seyreltme uygulaması sonrasında yaprak, sürgün ve meyvelerdeki besin elementi konsantrasyonlarının belirlenmesi hedeflenmiştir.

Anahtar Kelimeler: Antep fıstığı, Salkım Seyreltmesi, Şeker, Mineral Elementler.

EXTENDED ABSTRACT

Pistacia genus has 11 species, among these species only *Pistacia vera* L. has edible nuts and commercial value (Kafkas and Kaska 1997). Pistacia species are located in the Northern and Southern hemispheres between 30-45 latitude degrees and can be grown in microclimates suitable for the climatic conditions there (Bilgen, 1973). Pistachio has two gene centers, one of which is the Near East gene center, which covers the high places of Anatolia, the Caucasus, Iran and Turkmenistan, and the other is the Central Asian gene center (Ayfer, 1964). Turkey is located in the Near East gene center. Pistachio is a fruit that is delicious and rich in nutrients. Being rich in protein and other nutritional values makes pistachio more precious. Moreover, 100 grams of pistachios contain zero cholesterol. Besides, 100 grams of pistachio nuts provide 597 calories and it contains 19.3% protein, 53.7% fat and 19% carbohydrates and also rich in phosphorus, potassium, calcium and A, B1 and B6 vitamins. Pistachio has positive effects such as lowering the level of cholesterol in the blood, reducing the risk of coronary heart disease and preventing the increase in blood sugar. In addition, besides its nutritious feature, it is used not only as a snack but also as a raw material in sugar and sugar products industry (Yavuz, 2011). The variety which has been studied in this thesis was the “Kirmizi” which is early maturing (early September). Nut quality is close to the "Uzun" variety and it can be easily grown in places with a slightly shorter summer season than Gaziantep province. Since pistachio is a type of nut that shows alternate bearing phenomena, and so production varies according to years and countries. Respectively countries where pistachios are produced most, are Iran, the USA and Turkey. On average, 83.4% of the world's total pistachio production is provided by these countries. Iran is the leader in pistachio production. The USA has recently increased its production volume by developing production and irrigation techniques and has become a competitor to Iran. Turkey is one of the rare countries with a very high potential for horticultural production in the world and most of the fruit species can be grown in Turkey commercially. Turkey is the homeland of pistachio, the formation and development center of the cultivars and is one of the countries with important genetic resources. Samples of leaves, fruits and shoots from 30-year-old “Kirmizi” variety pistachio trees grown in Nizip region of Gaziantep, in 4 different periods from the beginning of May to the end of July from the thinning applied and non-applied branches were used. Applied trees were thinned 1 month before of first sampling date. A total of 72 samples were studied. Leaf, fruit and shoot samples of this variety, which were applied and non-applied were brought from Gaziantep to Adana province in the cold chain condition and after harvesting immediately treated with liquid nitrogen and dried by freeze-drying method (Ilshın FD5508 Bondıro Vacuum Freeze Dryer). All samples homogenized with a coffee grinder and stored at -80^{o}C until analyzed. Many fruit species require a reduction of fruit load to provide a vegetative equilibrium; normally this objective is achieved with the thinning operation. For this reason, thinning performed to provide optimum fruit load and quality. Thinning applications purposes 2 main goals, first provide high-quality products for first-class markets by producing fruits with desirable taste with optimum sugar content, optimum flesh firmness for appropriate storage length and sufficient size and coloration, second to overcome alternate bearing by providing regular crop load. However, although other cultural procedures are applied more or less, thinning is not applied adequately in our country. In this research main goal was to determine the effect of the flower cluster thinning application, on metabolic responses of pistachio (*Pistacia vera* L.cv. Kirmizi) depending on the fruit ripening stages. In this research, parameters such as phytohormones, sugar contents, mineral nutrients and semiquinone radicals were investigated. As a result of this study physiological parameters such as phytohormones, sugar content, mineral nutrients and semiquinone radicals have a strong effect on alternate bearing and fruit ripening.

GENİŞLETİLMİŞ ÖZET

Pistacia cinsine ait 11 tür vardır, bu türlerden sadece *Pistacia vera* L. yenilebilir kuruyemiş ve ticari değere sahiptir (Kafkas ve Kaska 1997). Pistacia türleri, Kuzey ve Güney yarım kürede 30-45 enlem dereceleri arasında bulunur ve buradaki iklim koşullarına uygun mikro klimalarda yetiştirilebilir (Bilgen, 1973). Antepfıstığı, biri Anadolu'nun yüksek yerlerini, Kafkaslar'ı, İran'ı ve Türkmenistan'ı kapsayan Yakın Doğu gen merkezi, diğeri ise Orta Asya'yı kapsayan iki gen merkezine sahiptir. (Ayfer, 1964). Türkiye Yakın Doğu gen merkezinde yer almaktadır. Antep fıstığı, lezzetli ve besin açısından zengin bir meyvedir. Protein ve diğer besin değerleri açısından zengin olması Antep fıstığını daha değerli kılar.100 gram Antep fıstığı sıfır kolesterol içerir. Ayrıca 100 gram antep fıstığı 597 kalori, %19.3 protein, %53,7 yağ ve %19 karbonhidrat içerir ve ayrıca fosfor, potasyum, kalsiyum ve A, B1 ve B6 vitaminleri açısından zengindir. Antep fıstığının kandaki kolesterol düzeyini düşürmesi, koroner kalp hastalığı riskini azaltması ve kan şekerinin yükselmesini engellemesi gibi olumlu etkileri vardır. Ayrıca besleyici özelliğinin yanı sıra sadece atıştırmalık olarak değil şeker ve şekerli mamuller sanayinde hammadde olarak da kullanılmaktadır (Yavuz, 2011). Bu tezde incelenen çeşit, erken olgunlaşan (Eylül başı) meyve kalitesi "Uzun" çeşidine yakın olan ve Gaziantep iline göre biraz daha kısa yaz mevsimi olan yerlerde rahatlıkla yetişebilen "Kırmızı" çeşididir. Antep fıstığı, periyodisite gösteren bir sert kabuklu meyve türü olduğu için üretim yıllara ve ülkelere göre değişiklik göstermektedir. Antep fıstığının en çok üretildiği ülkeler sırasıyla İran, ABD ve Türkiye'dir. Dünya toplam fıstık üretiminin ortalama %83.4'ü bu ülkelerden sağlanmaktadır. Fıstık üretiminde İran liderdir. ABD son zamanlarda üretim ve sulama tekniklerini geliştirerek üretim hacmini artırmış ve İran'a rakip olmuştur. Türkiye, dünyada bahçecilik üretimi için potansiyeli çok yüksek olan ender ülkelerden biridir ve Türkiye'de ticari olarak yetiştirilebilen meyve türlerinin çoğu bulunmaktadır. Türkiye fıstığın anavatanı, çeşitlerin oluşum ve gelişme

merkezi olup, önemli genetik kaynaklara sahip ülkelerden biridir. Antep fıstığı, Türkiye ekonomisinde büyük öneme sahip 10 üründen biridir. Bu araştırmada, Gaziantep'in Nizip yöresinde yetiştirilen 30 yaşındaki “Kırmızı” çeşidi fıstık ağaçlarından Nisan ayında seyreltme uygulanan ve uygulanmayan yaprak, meyve ve sürgün örnekleri mayıs başından temmuz ayı sonuna kadar 4 farklı dönemde alınan örnekler materyal olarak kullanılmıştır. Bu çeşidin çiçek salkımları seyreltilen ve seyreltilmeyen ağaçlardan alınmış yaprak, meyve ve sürgün örnekleri soğuk zincirde Gaziantep'ten Adana iline getirilmiş ve hasattan hemen sonra sıvı azot ile muamele edilerek dondurularak kurutma yöntemi ile kurutulmuştur (Ilshın FD5508 Bondiro Vakumlu Soğuk Kurutucu). Tüm örnekler bir kahve değirmeni ile homojenleştirilmiş ve analiz edilene kadar -80 °C'de muhafaza edilmiştir. Periyodisite eğilimi gösteren antepfısığı elma gibi pek çok meyve türünde, düzenli verim elde edebilmek için meyve yükünün azaltılmasını gerekmektedir. Normalde bu hedefe seyreltme işlemi ile ulaşılır. Bu nedenle optimum meyve yükü ve kalitesini sağlamak için seyreltme yapılır. Seyreltme uygulamalarının 2 ana amacı vardır, birincisi optimum şeker içeriğine sahip, istenen tada sahip, uygun depolama süresi için optimum meyve et sertliğine sahip ve yeterli büyüklük ve renklenmiş meyveler üreterek pazarlar için yüksek kaliteli ürünler sağlamak, ikincisi ise düzenli ürün yükü sağlayarak periyodisitenin üstesinden gelmektir. Ancak sulama, gübreleme, budama gibi bazı kültürel işlemler az çok uygulanmasına rağmen ülkemizde seyreltme yeterince uygulanmamaktadır. Bu araştırmada, çiçek salkımı seyreltme uygulamasının meyve olgunlaşma aşamalarına bağlı olarak Antep fıstığının (*Pistacia vera* L.cv. Kırmızı) farklı organlarında metabolik tepkilerine etkisinin belirlenmesi amaçlanmıştır. Bu araştırmada fitohormonlar, şeker içerikleri, mineral besin maddeleri ve semikinon radikalleri gibi parametreler araştırılmıştır. Bu çalışmanın bir sonucu olarak, fitohormonlar, şeker içeriği, mineral besinler ve semikunion radikalleri gibi fizyolojik parametreler, periyodisite ve meyve olgunlaşması üzerinde güçlü bir etkiye sahip olduğu sonucuna varılmıştır.

ACKNOWLEDGEMENTS

This research was carried out under the guidance of my major supervisors Prof. Dr. Ebru KAFKAS and Prof. Dr. Hab. Iwona MORKUNAS who have been a great help to me in performing my thesis analysis and writing my thesis. Especially Prof. Dr. Ebru KAFKAS for sharing her support and knowledge with me in all conditions. I would like to express my deepest appreciation to my second supervisor Prof. Dr. Hab. Iwona MORKUNAS has been the biggest support for me about my study and my life in a new country when I was miles away from my family. In addition, a thank you to Prof. Dr. Salih KAFKAS who support me to presents this study.

Also a big thanks to Erasmus Program and both side's coordinators who gave me opportunity to perform my thesis analysis in Poland where I met with valuable people. I thank all my committee members, Prof. Dr. Salih KAFKAS, Assoc. Prof. Dr. Murat GÜNEY and Asst. Prof. Dr. Hayat TOPÇU who spend their precious time to share their valuable ideas with me during committee meeting.

I would like to also thanks to Prof. NCU Dr Hab. Jacek KĘSY, Dr. Hab. Waldemar BEDNARSKI, Dr. Hab. Mateusz LABUDDA and Dr. Agnieszka WOŹNIAK for their intense help to perform my thesis analysis and writing stage.

I present my deepest appreciation for the big moral support given by my beloved uncle Ali Musa BOZDOĞAN and aunt Nigar YARPUZ BOZDOĞAN and my family.

Also i would like to thank Kasım Boler for his help in providing the thesis material.

Finally, I would like to thanks all of my labmates who helped me during my research work and thesis preparation. Especially Şule HİLAL and İpek DEĞİRMENCİ ÜRÜN who were not only labmates but also older sister for me.

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LIST OF ABBREVIATIONS

µ	: Micron
µl	: Microliter
ABA	: Abscisic Acid
ACC	: 1-aminocyclopropane-1-carboxylic acid
Al_2O_3	: Aluminium Oxide
B	: Boron
BA	: Benzyladenine
BHT	: Butylated Hydroxytoluene
Ca	: Calcium
CO_2	: Carbon dioxide
Cr3+	: Chromium (III)
Cu	: Copper
EPR	: Electron Paramagnetic Resonance
ET	: Ethylen
eV	: Electronvolt
FA	: Folic Acid
Fe	: Iron
G	: Gauss
GA3	: Gibberellic Acid
GAP	: Southern Anatolian Project
GCMS	: Gas Chromatography Mass Spectrometry
HCl	: Hydrochloric acid
HPLC	: High Pressure Liqiud Chromatography
IAA	: Indole-3-acetic acid
K	: Potassium
L	: Liter
Mg	: Magnesium

mg	: miligram
mm	: Milimeter
Mn	: Manganese
Mn^{2+}	: Manganese(2+)
mW	: Megawatt
N	: Nitrogen
NAA	: Naphthalene Acetic Acid
NAD	: Naphthaeneacetamide
P	: Phosphorus
RID	: Refractive Index Detector
SIM	: Selected Ion Monitoring
Zn	: Zinc

1. INTRODUCTION

Pistachios are known as "Green Gold" in Turkey, especially in the Middle East region and provide important revenue for producers (Gündeşli et al, 2018). Pistacia genus has 11 species, among these species only *Pistacia vera* L. has edible nuts and commercial value (Kafkas and Kaska 1997). It was reported by Plinius who is governor of Syria that the pistachio was first cultured in South Anatolia, where the Eti settled and was taken to Rome by the Syrian governor Vitellus in the first century AD. It is stated that based on the archaeological excavations carried out in Turkey, pistachio fossils dating back at least seven thousand years ago were found and this fruit was consumed by people at that time (Avanzato and Vasallo, 2008).

Pistacia species are located in the Northern and Southern hemispheres between 30-45 latitude degrees and can be grown in microclimates suitable for the climatic conditions there (Bilgen, 1973). Pistachio is the origin of two, one of which is the Near East gene center, which covers the high places of Anatolia, the Caucasus, Iran and Turkmenistan, and the other is the Central Asian gene center (Ayfer, 1964). Turkey is located in the Near East gene center.

The Place of Pistachio in Systematic

- Order: *Sapindales*
- Family: *Anacardiacea*
- Genus: *Pistacia*
- Species: *Pistacia vera* L. (Tekin et al, 2001).

Propagation status of Pistacia species in the Southeastern Anatolia Region during recent surveys: *P. vera* L., *P. terebinthus* L., *P. khinjuk* stock species and new hybrids resulting from their foreign fertilization are abundant (Bilgen, 1973). Melengiç (*P. terebinthus*) is mostly used in Gaziantep, Adıyaman, Kahramanmaraş and Şanlıurfa provinces; buttum (*P. khinjuk*) in Siirt, Hakkari, Gaziantep, Adıyaman and Bitlis provinces; wild pistachio (*P. vera* and hybrids) are located in Gaziantep and Kahramanmaraş provinces (Arpacı et al,1997) (Figure 2) (Turkey Map).

Figure 1.1. Map of provinces in Turkey

Pistachio is a fruit that is delicious and rich in nutrients. Being rich in protein and other nutritional values makes pistachio more precious. Moreover, 100 grams of pistachios contain zero cholesterol. Besides, 100 grams of pistachio nuts provide 597 calories and it contains 19.3% protein, 53.7% fat and 19% carbohydrates and also rich in phosphorus, potassium, calcium and A, B1 and B6 vitamins. Pistachio has positive effects such as lowering the level of cholesterol in the blood, reducing the risk of coronary heart disease and preventing the increase in blood sugar. In addition, besides its nutritious feature, it is used not only as a snack but also as a raw material in sugar and sugar products industry (Yavuz, 2011). Pistachio has become a valuable product in domestic and foreign markets due to its high nutritional value, tasteful consumption and limited production area in the world (Altuntaş and Mutlu 2007).

In Turkey 'UZUN' variety is widely grown and it is followed by Siirt, Kırmızı, Halebi and Ohadi varieties.

Table 1.1. Commonly grown pistachio varieties in Turkey and its some properties

Varieties	Fruit Size (pc/100g)	Cracking Rate	Yield(%)
Barak yıldızı	69	98.0	44.0
Tekin	86	31.3	35.3
Halebi	70	95.3	42.2
Kırmızı	83	76.0	38.6
Ohadi	78	90,7	38.4
Siirt	77	78.7	38.7
Uzun	70	76.7	46.5

Characteristics of some commonly grown pistachio varieties;

Tekin: It has a high-quality variety than Siirt, which is the highest quality variety in Turkey. It can be grown in places where the summer season is long and hot as Gaziantep province.

Barak Yıldızı: It is the earliest variety in Turkey. It has dark green fruit and ripens in Gaziantep province in first or second week of August. It can also be grown in the highland areas, where the summer season lasts a short time.

Kırmızı: Our early maturing (early September) fruit quality is close to the "Uzun" variety. It is not as common as the "Uzun" variety. It can easily grow in places with a slightly shorter summer season than Gaziantep province.

Halebi: Ripening of this variety is in mid-season (mid-September), fruit quality better than "Uzun" and "Kırmızı" variety. However, it’s not so popular as these varieties. It is used as a raw fruit to both nuts and food industry.

Uzun: It is a variety that ripens in the middle season (mid-September) and has medium fruit quality traits. Its yield is higher than “Halebi and” “Kırmızı” varieties. Despite its small in size, it is known all over the world due to having excellent taste it has been mostly preferred especially in Gaziantep region in Turkey where a common growing place. Ripening time is later than other varieties, but yield is higher. It is very common in food industry and it is the mostly preferred and common variety in Turkey.

Ohadi: The fruit quality is slightly better than the Siirt variety, but the yield is low and the tree development is weak. Its nuts ripen in late season (beginning of October) similar to the “Siirt” variety.

Siirt: It has 30% more yield than “Uzun” variety in wet and dry conditions. At the same time, alternate bearing tendency is lower than Uzun and Kırmızı varieties.

Pistachio trees are highly tolerant for adaptation in various climatic changes and highly profitable for farmers especially Southeastern Anatolia Region, where pistachio is produced extensively. Especially in this region, cultivation is carried out in completely dry conditions and mostly in barren, stony and sloping lands (Ertürk et al 2015). One of the important factors that determine the pistachio growing areas is temperature. In the summer, they need a lot of long-term high temperatures for the development and ripening of the fruit and a low temperature for a certain time in the winter. Especially, where the sum of the temperature is not enough, the fruits cannot fill them completely, the hard shell does not crack and the outer shell does not easily separate from the hard shell (Tilkat et al 2012).

Turkey is one of the gene centers of the species of the Pistacia genus. For this reason, pistachio nuts show a great variation in Turkey.

However, in the USA and Iran, all cultivation is carried out in irrigated conditions and fertile lands. With the GAP (Southern Anatolian Project), the production area can be irrigated, when it is irrigated yield and nut quality effected positively particularly suitable varieties and pollinators are preferred. In addition, in this region, technological developments are increasingly used in the processing industry, the methods of combating diseases and pests, pruning and fertilizer applications are increasingly continuing in the new orchards but still not at the desired level.

1.1. Pistachio growing in the world

Since pistachio is a type of nut that shows alternate bearing phenomena, and so production varies according to years and countries. Respectively countries where pistachios are produced most, are Iran, USA and Turkey. On average, 83.4% of the world total pistachio production is provided by these countries. Iran is the leader in pistachio production. The USA has recently increased its production volume by developing production and irrigation techniques and has become a competitor to Iran.

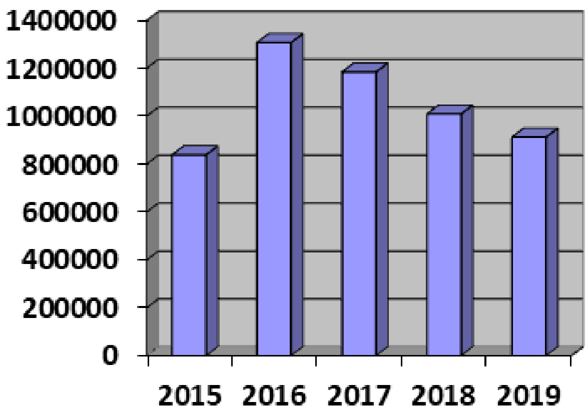

Figure 1.2. World pistachio production values of recent years (tons) (Anonymous, 2021a)

Pistachio production values increase every year in the world and Turkey. World pistachio production, which was 837,888 tons in 2015, reached approximately 911,829 tons in 2019 (Figure 1). Pistachio is among the top 5 in world nut production.

Table 1.2. Pistachio production quantities in major countries between 2014-2019 (Tons) (Anonymous 2021b)

Production	2014	2015	2016	2017	2018	2019
Iran	440814	430000	574987	648934	143695	337815
USA	233146	122470	406646	272290	447700	335660
Turkey	80000	144000	170000	78000	240000	85000
Syria	28786	28800	28800	51048	61631	31813
China	78232	83533	90310	95591	100873	106155
World	**887489**	**837888**	**1306137**	**1183602**	**1008479**	**911829**

As seen in Table 1.2., Iran is the biggest producer country in the world pistachio production. Iran provides 37.05% of the total world production with its 337,815 tons of production. USA follows Iran in terms of pistachio production. 98% of pistachio production in the USA is predominantly provided by the state of California. China is also one of the important producers in terms of production area and quantity. However, pistachio grown in China comes from a different species (*Pistacia chinensis*) and the nuts of this species are not suitable for edible consumption but this type of product is provided for biodiesel fuel production (Yavuz 2011). Turkey is the country that produces the most delicious pistachios in the world after Iran and the United States (Kızılgöz et al, 2009). When the 2019 data are analyzed 9% of total production was provided by Turkey. Turkey has a special location as it is located in the gene center of pistachio, and rich potential in terms of wild tree quantity and is not so selective in comparison to other fruit types in terms of soil and climate demands (Tunalıoğlu ve Taşkaya 2003; Gundesli et al. 2018).

1.2. Pistachio growing in Turkey

Turkey is one of the rare countries with a very high potential for horticultural production in the world and most of the fruit species that can be grown in Turkey commercially. Turkey is the homeland of pistachio, the formation and development center of the cultivars and is one of the countries with important genetic resources. Pistachio is one of the 10 products that are of great importance in the Turkish economy. According to data from the year 2020, a total of 77 thousand trees in the pistachio orchards 350 000 ha area in Turkey and 70% of them are able to occur fruit. With the desired increase in the number of trees in recent years, it is seen that the production will increase significantly in the coming years after the trees reach the efficiency age with the transition to closed irrigation cultivation established with the GAP (Southeast Anatolia Project) region. According to 2018 figures in Turkey, pistachio cultivation is carried out in an area of 350000 ha, and pistachio is ranked third in the world with 240.000 tons after Iran and the USA (Table 3). As seen in Table 3, when the data of pistachio production in Turkey is analyzed in the last 11 years, it is seen that there is a fluctuating change between 78.000 and 296.376 tons.

Although pistachio is grown in 56 provinces of Turkey, economical cultivation is carried out in Gaziantep, Şanlıurfa, Siirt, Adıyaman and Kahramanmaraş provinces. According to 2018 data, 95.25% of pistachio collective fruit gardens and 95.53% of fruit-bearing age trees are found in these provinces, while these provinces meet 95.64% of total production (Tables 3 and 4).

Table 1.3. Pistachio Production of Turkey between the years 2010-2020 (Anonymous 2021a)

Year	Production (Ton)	Quantity of Trees Which Can Give Fruit	Quantity of Trees Which Can't Give Fruit	Total Tree Quantity
2010	128000	29617	10562	40179
2011	112000	30868	10419	41287
2012	150000	37150	12428	49578
2013	88600	38116	12006	50122
2014	80000	39330	11153	50482
2015	144000	40597	11633	52230
2016	170000	42570	17193	59762
2017	78000	47766	19460	67225
2018	240000	49558	20529	70087
2019	85000	52061	20984	73045
2020	296376	54548	22722	77270

Table 1.4. The area and number of trees of pistachio orchards in the main provinces in 2017 and 2018 (Anonymous 2021b)

Provinces	Orchard (Decares)				Quantity of Trees Which Can Give Fruit			
	2017	%	2018	%	2017	%	2018	%
Gaziantep	1.363.473	41.46	1.363.473	38.46	17.703.343	37.06	17.991.655	36.30
Şanlıurfa	1.253.983	38.13	1.392.160	39.27	18.300.486	38.31	18.552.471	37.43
Siirt	188.073	5.71	282.071	7.95	2708000	5.66	5.318.953	10.73
Adıyaman	258.092	7.84	263.928	7.44	4.668.830	9.77	4.663.970	9.41
Kahramanmaraş	67.990	2.06	75.289	2.12	810.050	1.69	816.700	1.64

Table 1.5. Pistachio production values in the main provinces in 2017 and 2018 (Anonymous 2021b)

Provinces	Production (ton)			
	2017	%	2018	%
Gaziantep	14.762	18.92	90.183	37.57
Şanlıurfa	28.507	36.54	100.107	41.71
Siirt	7.944	10.18	11.301	4.70
Adıyaman	10.440	13.38	24.015	10.00
Kahramanmaraş	3.704	4.74	3.953	1.64

Pistachio is one of the most important and valuable nuts grown in Turkey. But when the number of pistachio trees and production amounts of the last 10 years are analyzed, it is seen that there is a fluctuating change in production and the increase in production is not sufficient. Alternate bearing phenomena in pistachio is one of the most important issues causing these fluctuations in trees production (Gündeşli 2017; Gündeşli et al, 2019). Alternate bearing is defined as the fact that the trees do not bear every year regularly so they called “OFF” year and “ON” year. Alternate bearing phenomena is an important problem seen in many varieties of horticultural plants, especially pistachios, olives, apples, etc. For this reason, Alternate bearing phenomena is important for both producer and consumer. Since this change in the amount of crops seen between the years coincides with the same period in the region, the producers cannot harvest the crops regularly every year, especially in the year of yield, prices fall down and this fact affect our manufacturers economically. In terms of consumer, while it faces a higher price in "OFF" year, and people can not buy due to the high prices. This situation also affects domestic and foreign trade balance negatively. Unlike other fruit alternate bearing in pistachio, there are plenty of flower buds occur every year. However, when the nut starts to fill the inside in the summer season flower buds fall out. For this reason, alternate bearing phenomena in pistachio occurs as a result of abscission of flower buds (June-July) instead of the absence of flower buds in the "OFF" year (Crane 1971; Brown et al, 1995; Lovatt and Ferguson, 2002; Kafkas et al, 2017). Different researches have been carried out for years in order to solve the problem of flower bud abscission in pistachio, but its effects on flower bud abscission alternate bearing mechanism in pistachio have not been determined exactly. The flower bud, which will bear fruit in the "ON" year in pistachio in the following year, mostly fall in July and August, during this period, due to the rapid development of the nut development inside, it competes with the flower buds by attracting nutrients.

1.3. Flower bud abscission and fruit ripening

It is known that flower bud abscission occurs as a result of the competition that embryo development of nut becoming more dominant and winning the race of using plant nutrients and carbohydrates (Crane, 1971; Nizima, 1997; Rosecrance, 1998; Lovatt and Ferguson, 2002; Talaie et al, 2003; Çetinkaya, 2004; Baninasab and Rahemi, 2006; Gundesli et al, 2019). In addition, some researchers working on this subject reported that as a result of the lack of plant nutrition and balanced can cause negative results such as; inadequate plant growth, low efficiency and quality impairments (Crane, 1971; Porlingis, 1974; Uriu and Crane 1977).

Fruit ripening is a stage that has paramount importance for foodstuff processing since nutritional composition of fruit is highly influenced by maturity. In fact nutritional values of mature fruit and unripe fruits show big difference such as higher amounts of free carbohydrates and lower amounts of amino acids and organic acids (Sciubba F. et al. 2017).

1.3.1. Effects of free radicals in the flower bud abscission and fruit ripening

Biotic and abiotic stresses are important conditions which affect flower bud abscission and fruit ripening. To overcome these stress conditions increased generation of free radicals plays a significant role. Moreover, stresses caused by applied cultural applications on plants may also activate the production of relatively stable free radicals, such as semiquinone radicals as a defense reacts on plant physiology (Morkunas, and Bednarski, W. 2008; Durak, et al 2019).

1.3.2. Effects of abscisic acid in the lower bud abscission and fruit ripening

Flower bud abscission is a physiological process, which regulated actively in response to exogenous conditions such as environmental parameters and endogenous stimuli such as phytohormones. Analysis showed that exogenous abscisic acid (ABA) significantly accelerated the transcriptional activity of the ET biosynthesis genes and strongly increased the level of ACC (1-aminocyclopropane-1-carboxylic acid). As a result, ABA has high importance to flower bud abscission by enhancing the ET biosynthesis rate (Wilmowicz, et al 2016).

1.3.3. The role of sugars in the flower bud abscission and fruit ripening

Sugar content can affect abscission in two ways; high carbohydrate level makes thicker cell walls and as a result of this abscission becomes more difficult and transportation of carbohydrates to ovularies supports embryo development and embryos produce more auxin which in turn retards abscission. Moreover carbohydrate level in abscission zone shows direct effect to abscission (Addicott et al., 1955).

1.3.4. Effects of mineral nutrition elements to the flower bud abscission and fruit ripening

Adding mineral elements to soil is a common way to improve plant growth for more than 2000 years. Increasing the available mineral nutrients in *Pisum sativum* decreased flower bud abscission by 75%. In citrus, a higher rate of flower abscission was correlated with a lower level of micronutrients such as zinc (Van Doorn and Stead, 1997).

1.4. Importance of thinning on fruit trees

Pistachio trees exhibit some physiological disorders such as abscission of inflorescence buds, fruit abscission, the production of blank, non-split, early-split and deformed nuts. Several studies show that mechanical or chemical fruit and flower thinning has direct effects on fruit quality parameters such as size, color, firmness, sugar and acid content. However thinning applications can show different results depending to crop load, age of wood, flower bud quality and competition within-cluster and canopy (Link, 2000). Many fruit species require a reduction of fruit load to provide a vegetative equilibrium; normally this objective is achieved with the thinning operation. For this reason, thinning performed to provide optimum fruit load and quality. Thinning applications purposes 2 main goals, first provide high-quality products for first-class markets by producing fruits with desirable taste with optimum sugar content, optimum flesh firmness for appropriate storage length and sufficient size and coloration, second to overcome alternate bearing by providing regular crop load. Removing of complete flower clusters is a method of orchard management during the high yield season of a pistachio producer, aim of this application prevent intense flowering and alternate bearing. In modern fruit growing, cultural practices are very important to obtain high-quality and fruitful fruit. Thinning is at the top of these cultural practices. In addition to obtaining larger, homogeneous and well-colored fruits by thinning application as well as ensuring a balanced product distribution on the tree, branch breakage is prevented, periodicity tendency is reduced and trees have increased resistance to winter cold (Akgül, 2003; Butar et al, 2016). In addition, plant protection against diseases and pests becomes easier, and processing like harvesting and classification expenses are decreasing (Yıldırım and Koyuncu 2004). Researchers reported that cultural applications like fruit thinning in pistachio reduces the fall of flower buds (Crane et al 1973). However, although other cultural procedures are applied more or less, thinning is not applied adequately in our country.

The main goal of this thesis was to examine the effect of the flower cluster thinning application, on molecular and metabolic responses of pistachio (*Pistacia vera* L.cv. Kirmizi) depending on the time that has elapsed since making the flower cluster thinning. The first objective was to determine whether the above treatment alters the generation of semiquinone radicals in pistachio organs (fruits, leaves and shoots). The second objective was to verify whether it causes elevated generation signaling molecules, i.e. phytohormones such as abscisic acid (ABA), salicylic acid (SA) and indole-3-acetic acid (IAA) and changes in the sequence/period of their generation, and in the transduction of signals from organ to organ. Moreover, the third objective was to determine whether changes in the sugar levels occur after the flower cluster thinning. Additionally, it was aimed to determine the nutrient element concentrations in leaves, shoots and fruits after the flower cluster thinning application in the "Kırmızı" pistachio variety, which is one of the most important varieties in Turkey and especially in the Southeastern Anatolia region, showing absolute alternate bearing phenomena, from blooming to fruit harvesting.

2. LITERATURE

Crane et al. (1973), reported that flower buds were shed in the period when they formed abundant flower buds every year, unlike other fruit types that showed periodicity, but when the product was in excess. For this reason, alternate bearing phenomenon in pistachio has been reported as a result of abscission of these buds, rather than the absence of flower buds.

Lovatt et al. (2002), reported that excessive flower-bud abscission began in June in the "ON" year, and the time for domestic nut development increased throughout July, causing it to become "OFF" in the following year. Although the unique mechanism causing periodicity in pistachio has been defined, while its physiological basis has not been defined yet.

2.1. Importance of Plant Growth Regulators on Alternate Bearing

Crane and Nelson (1971), stated that alternate bearing phenomena in pistachio trees may be occurred due to the lack of carbohydrate biosynthesis as products of CO_2 assimilation of which (sugars) are one of the internal factors to the flower bud abscission seen in June.

Crane et al. (1973), reported that fruit breakage and bracelet removal practices in pistachio reduced the shedding of flower buds.

Weinbaum et al. (1994), reported that the trees needed nitrogen and carbohydrates for the development of nut kernel in the “ON” year of pistachio.

Lovatt and Ferguson (2002), indicated that the special temporal relationships between the beginning of flower bud and shoot growth in pistachio are responsible for the parts of fruit development, especially flower development, during the year when it gives a severe product in pistachio. The flower buds that will bear fruit next year, mostly fall in July and August, the fruit develops faster in this period, and the fruit is directed towards maturity, flower bud abscission occurring the competition with the flower buds, and in this case, the carbohydrate deficiency occurs in the bud castings as a result of the fact that the flower bud abscission occurs.

2.2. Importance of Plant Nutrients on Alternate Bearing

Porlingis (1974) stated that flower bud abscission in pistachios during the growth of the pericarp, but the castings begin to fill and develop inside the fruit and appear within 20 days after that, the seed contains approximately 4% N, 30 days after full bloom, 2.5% N is found in the pericarp, 1.5% N after 70 days. It was also determined that the content of N in the pericarp decreases up to 1% 30 days before fruit ripening and that the N in the pericarp can be carried towards the fruit during the growth of the fruit. According to the researcher, 103 days after the full bloom (August 10), it was found that trees containing 0.69 mg N did not pour off the buds of the fruit-bearing trees and that those containing N contained pouring, and the buds of trees that did not bear fruit contained 0.73 mg N.

Talaie et al. (2003), investigated the effect of soluble carbohydrates on flower bud abscission in “Ohadi” pistachio cultivars. According to the study, they determined that the relationships between soluble carbohydrate concentration and time in flower bud abscission are important, but it was found that the effect of soluble carbohydrate concentration on alternate bearing was not important. According to the study, a decrease in soluble carbohydrate concentration was observed in June-July, and they stated that this situation caused flower bud abscission. However, they determined that there was an increase in carbohydrate concentration during this period in the “OFF” year.

Baninasab and Rahemi (2006), informed that during the summer growth and development of pistachio nuts, they examined their flower bud abscission in the year of ‘ON’ and year of ‘OFF’. Researchers have determined the content of carbohydrates (fructose, glucose, sucrose and starch) in roots, one-year shoots, leaves, flower buds and fruits between flowering and harvesting in the 30-year-old “Ohadi” pistachio variety to investigate the role of carbohydrates in flower bud abscission. According to the study, the amount of soluble sugar and starch existed up to 70 days after flowering and were relatively similar in many organs of the “off” year trees. On the other hand, during the fruit development, there was a decrease in the soluble sugar and starch content in many organs during the fruit development. In the same period, it was observed that soluble sugar and starch began to accumulate in more concentrations in various organs of the “OFF” year and the trees in the “ON” year exceeded the measured amount.

Ferguson et al. (2006), stated that it is not known what determines the percentage of fruit formation, but that the tree's stored carbohydrate capacity can be caused by determining the percentage of fruits that are formed first. The researchers stated that this theory was confirmed by proving higher fruit percentages in diluted clusters. However, they reported that diluted and not diluted inflorescences have almost the same number of fruits, but diluted inflorescences can have larger fruits.

Okay et al, (2011), reported that leaf samples of “Kırmızı” variety showed different changes in the “ON” and “OFF” years. According to the study abscisic acid was observed at the highest level (121.683 mg/g^{-1}) in “ON” year in May and it showed regular decrease. The lowest level of abscisic acid (6.496 mg/g^{-1}) was determined in the following “OFF” year in May. In the following “ON” year abscisic acid which was observed highest level (126.087 mg/g^{-1}) in July decreased to the lowest level (6.565 mg/g^{-1}) in September. The abscisic acid amount was high in “ON” year and low in “OFF” year.

Çetiner, (2015), investigated macro and micro nutrients in the leaves of different pistachio varieties grown in the town of Şanlıurfa Akçakale in Turkey. According to the researcher, the varieties of 'Siirt' were found to be N 1.747%, P 0.084%, K 0.0509%, Mg 0.470%, Ca 2.042%, Fe 155.73 ppm, Cu 13.387 ppm, Zn 12.053 ppm, Mn 34.20 ppm, B 138.26 ppm. They determined that the variety of 'Ohadi' was N 1.711%, P 0.078%, K 0.457%, Mg 0.528%, Ca 2.514%, Fe 150.36 ppm, Cu 10.617 ppm, Zn 12.460 ppm, Mn 46.41 ppm, B 134.50 ppm. In the 'Kerman' cultivar, N was determined as 1.743%, P 0.083%, K 0.478%, Mg 0.527%, Ca 2.500%, Fe 191.46 ppm, Cu 11.290 ppm, Zn 9.749 ppm, Mn 41.32 ppm, B 126.56 ppm.

Gündeşli et al. (2018), carried out to determine the periodic variation of macro elements in the cluster and nuts of the "ON" year cultivars in the "Uzun" pistachio cultivar after full blooming, the nitrogen content of the clusters in 2015 was ranged 0.97%-2.40% and 1.64-2.23% in 2016. In the early periods of vegetation, the nitrogen content was very high in 2015 and 2016 years, but also in the 2015, more nitrogen content was determined compared to 2016. When the phosphorus contents of the cluster and nut samples are analyzed, it was found 0.06.%- 0.37% in the clusters of the 2015 year, while it was found 0.05-0.22% in the clusters of the 2016 year, in the nuts were ranged between 0.15% to 0.30% in 2015 and 0.11% to 0.35% in 2016. They reported that the phosphorus content, which was high in the early vegetation period in both years, decreased until the harvest time. When the potassium contents are examined, the highest potassium content was found 75 days after full blooming with 1.80% in the clusters of 2015, and 1.35-1.80% was found in the clusters of 2016. When the calcium content of the clusters of different periods is examined, the shoots of the 2015 year were found to be 0.24-0.43%, while the clusters of the 2016 year were found to be 0.21-0.33%. When the magnesium content of cluster samples taken from different physiological periods is analyzed, the value of 0.02-0.14% in the clusters of 2015, has been in the range of 0.053-0.12% in the shoots of 2016 year.

2.3. Importance of Thinning

Boler, (1997), carried out research in a commercial orchard for three years to determine the effects of flower bud thinning and pruning on alternate bearing and nut quality. Kirmizi and Keten Gomlek varieties were used in both experiments. As a result of both treatments, treated trees had positive effects such as high yield and good nut quality of pistachios were obtained. Bigger nuts with higher proportion of split nuts were obtained in thinned trees, also.

Ramezanian A., and Rahemi M. (2007), performed 2 type of thinning to determine effects of flower bud retention, the first year different doses of ethephon, urea, naphthaleneacetic acid (NAA) and naphthaleneacetamide used as chemical thinning method by spraying on two branches of nine uniform trees with four replications. During the second year, hand thinning treatment was used in addition to the previous chemical thinning. Chemical treatments reduced flower bud abscission; among them, ethephon treatments were the most effective on flower bud retention. As compared with other treatments, hand thinning treatments had also positive effect on flower bud retention. Fruit thinning also increased kernel weight, reduced the number of nuts per ounce and decreased blank fruits.

Zarei et al. (2014), investigated the effects of chemical thinning on seasonal variations of leaf and fruit mineral compositions of "Soltani" apple trees. Treatments were applied when fruit diameters were about 8 to 10 mm with following doses; un thinned control, naphthalene acetic acid (NAA) at 5 and 10 mg L^{-1}, naphthaleneacetamide (NAAm) at 25 and 50 mg L^{-1}, benzyladenine (BA) at 50 and 100 mg L^{-1} and carbaryl at 500 and 1000 mg L^{-1}. As a result, chemical thinners affected leaf concentrations of N and K but not P, Ca and Mg. Chemical thinning increased the fruit's P, K and Mg concentrations. It is concluded that fruit chemical thinning may affect the accumulation of some nutrients, especially P and K in apple fruits and could improve fruit quality as well.

Kamiab et al. (2020),, performed different thinning methods to overcome alternate bearing in pistachio. Three chemical thinners including gibberellic acid (GA_3; 50, , and ethephon (75, 150 and 200 mg/L), and hand thinning (20%) were applied in a commercial homogenous orchard of pistachio. The research was carried out in the "Akbari" pistachio variety 2 weeks after full bloom during 2017–2018 in Rafsanjan region in Iran. Ethephone at 200 mg/L and NAA at 120 mg/L caused the highest fruit abscission. Hand thinning, NAA (120 mg/L) and all ehtephone treatments decreased fower bud abscission by about 30% in the "ON" year. Vegetative traits such as shoot length and leaf area showed significant increase under the treatments. Chemical thinning remarkably enhanced total non-structural carbohydrate, especially in "ON" year. All of the treatments except GA_3 increased tree yield in the "OFF" year. Qualitative fruit characteristics such as nuts per ounce, blank and split nuts percentage improved. Alternate bearing in pistachio tree was adequately controlled and chemical thinning was more effective than hand thinning.

3. MATERIAL AND METHODS

3.1. Material

Samples of leaves, fruits and shoots from 30-year-old "Kirmizi" variety pistachio trees grown in Nizip region of Gaziantep, in 4 different periods from the beginning of May to the end of July from the thinning applied and non-applied branches were used. Applied trees were thinned 1 month before first sampling date. A total of 72 samples including leaf, fruit and shoot samples of this variety was studied. The samples applied and non-applied thinning applications were taken and brought from Gaziantep to Adana province under the cold chain condition and after harvesting immediately treated with liquid nitrogen and dried by freeze-drying method (Ilshın FD5508 Bondıro Vacuum Freeze Dryer). All samples were homogenized with a coffee grinder and stored at -80 ^{o}C until analyzed. The analyzes were carried out in the Plant Nutrition Physiology and Chromatography Laboratories of the Cukurova University Faculty of Agriculture, Department of Horticulture in Turkey and Department of Plant Physiology, Faculty of Agronomy and Bioengineering of Poznań University of Life Sciences, Institute of Molecular Physics Polish Academy of Sciences in Poznań and Department of Plant Physiology and Biotechnology, Faculty of Biological and Veterinary Sciences of Nicolaus Copernicus University in Poland.

Figure 3.1. Homogenization stage of dried materials

3.1.1. Kirmizi Variety

"Kirmizi" is one of the important commercial variety which produced in Turkey mostly. It mainly grows in the high-altitude parts of the Gaziantep region and is preferred due to its early variety (Polat et al., 2007). It is a variety that ripens early (beginning of September) and its fruit quality is close to the "Uzun" variety. It is not as common as the "Uzun" variety. It can be grown easily in places with a slightly shorter summer season than Gaziantep.

Figure 3.2 Nuts of Kırmızı variety

3.1.2. Climatic Data of Research Area

Climatic data for the research area was obtained from the General Directorate of Meteorology. Monthly and yearly average temperatures, maximum and minimum temperatures, the average hours of sunshine and mean rainfall values in the period of 2018-2019 years for Gaziantep province are given in Table 3.1.

Table 3.1. Average climatic values obtained by 2018-2019 growing period in Gaziantep region in Turkey (Anonymous, 2021)

Months	**Average Temperature (° C)**	**Average Maximum Temperature (° C)**	**Average Minimum Temperature (° C)**	**Average Hours of Sunshine (Hours)**	**Total Rainfall (mm)**
January	3.0	7.4	-0.7	3.6	102.8
February	4.4	9.3	0.1	4.4	83.4
March	8.1	13.8	2.9	5.5	72.5
April	13.3	19.6	7.2	7.0	52.7
May	18.7	25.4	11.8	8.4	31.3
June	24.2	31.1	17.0	10.3	8.5
July	27.9	35.1	21.0	10.6	7.1
August	27.7	35.2	20.9	10.0	5.2
September	23.3	31.0	16.1	8.7	7.2
October	16.6	24.1	9.9	6.9	37.3
November	9.8	16.2	4.4	5.3	62.0
December	4.9	9.6	1.0	3.5	98.5
Annual	15.2	21.5	9.3	84.2	568.5

3.2. Methods

3.2.1. Detection of phytohormone abscisic acid (ABA)

Samples (leaves, shoots and fruits) of *Pistacia vera* L. were homogenized in liquid nitrogen and extracted twice with 20 mL 80% (v/v) methanol containing 20 mg/L butylated hydroxytoluene (BHT). Subsequently, 100 ng of deuterated ABA, [2H6] ABA, were added as internal standard. The mixture was shaken overnight. The extract was then centrifuged and the supernatant was collected in evaporation flasks. For an additional centrifugation step, the remaining pellet was resolved two times in 10 ml of 80% (v/v) methanol, shaken for two hours and centrifuged again. This supernatant was then added to the evaporation flasks. Next, the extract was evaporated until all methanol was removed. The flasks were washed with additional H_2O and the extract was collected in centrifugation tubes. The appropriate amount of HCl was added for acidification until pH 2 was reached. This was checked with coloured pH test strips. Next, the extract was centrifuged and the supernatant was collected into Erlenmeyer for solvent partition. The supernatant was partitioned three times against ethyl acetate. The organic phase was collected in evaporation flasks and dried under vacuum. The dry residue was then resolved in 5 ml 1 M formic acid (FA) and loaded on a Discovery® DSC- 18 SPE cartridge (Supelco Inc., USA). These cartridges (columns) were preconditioned with 4 ml methanol and allowed to equilibrate with 4 ml of 1 M FA. The columns were then subsequently washed with 4 ml 1 M FA, 4 ml of 20 % methanol in 1 M FA and finally phytohormones were eluted with 4 ml of 80% methanol in water. The eluate was evaporated to dryness, resolved in 200 µl of 20% acetonitrile in water and applied to HPLC for further sample purification. HPLC was done with a SUPELCOSIL ABZ+ PLUS column (250 x 4.5 mm, 5 µm particle size; Supelco). The samples were chromatographed with a linear gradient of 20-80% acetonitrile in 0.1 M FA in 20 min, flow rate of 1.0 ml/min at temperature 22°C. The fractions collected at 12 ± 0.5 min were evaporated to dryness and transferred to appropriate small glass tubes with 100 % methanol. The methanol was then evaporated using N2. Next the residue was methylated with diazomethane dissolved in diethylether. After this, the diethylether was evaporated with N2 and the residue was dissolved in 30 µl of 100% methanol. 1 µl of this sample was analyzed by gas chromatography (GC)/mass spectrometry (MS) (Auto-System XL coupled to a TurboMass, Perking-Elmer) using a MDN-5 column (30mm x 0.25 mm, 0.25 µm phase thickness; Supelco). The GC temperature program was set at 60 °C for 1 min, 60-250 °C at 10 °C/min, flow rate 1.5 ml/min, injection port was 280 °C, electron potential 70 eV. The retention times of ABA and [2H6]ABA were 14.07 and 14.3 min, respectively. GC/MS was performed by monitoring mass. GC/MS–SIM was performed by monitoring m/z 190 for endogenous ABA and 194 for [2H6] ABA, 130 for endogenous IAA and 132 [2H2] IAA according to the method described by Vine et al. (1987).

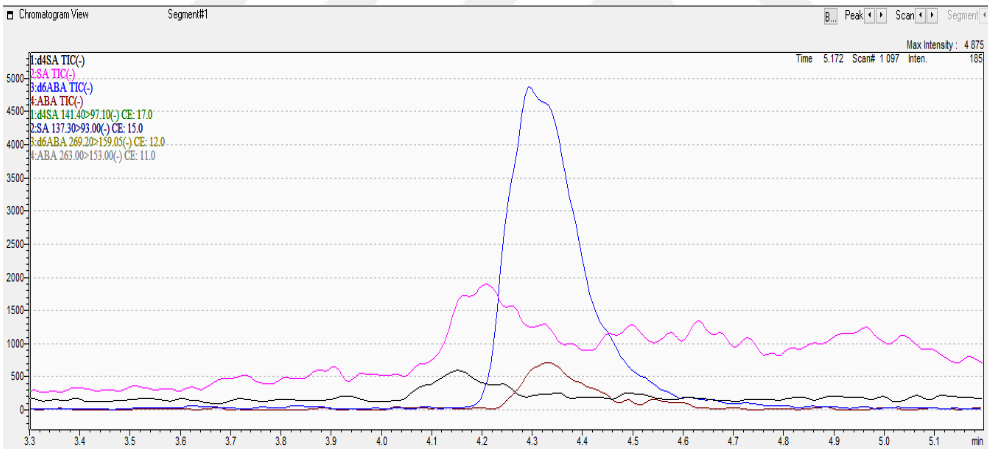

Figure 3.3. Chromatogram View of ABA Analysis.

3.2.2. Electron paramagnetic resonance (EPR)

Samples (leaves, shoots and fruits) of *Pistacia vera* L. were lyophilized and lyophilized plant material was transferred to EPR-type quartz tubes of 4 mm in diameter. Electron paramagnetic resonance measurements were performed at room temperature with a Bruker ELEXSYS X-band spectrometer. The EPR spectra were recorded as the first derivatives of microwave absorption. A microwave power of 2 mW and a magnetic field modulation of about 2 G was used for all the experiments to avoid signal saturation and deformation. EPR spectra of free radicals and Mn^{2+} ions were recorded in the magnetic field range of 1400-4200 G. In order to determine the number of paramagnetic centers in the samples, the spectra were double-integrated and compared with the intensity of the standard Al_2O_3:Cr3+ single crystal with a known spin concentration (Bednarski et al., 2010; Mai et al., 2013; Morkunas et al., 2003, Morkunas and Bednarski, 2008, Morkunas et al., 2004, Morkunas et al., 2008, Morkunas et al., 2013). Before and after the first integration some background corrections of the spectra were made to obtain a reliable absorption signal before the second integration. Free radicals gave signals with one g-values of 2.0045 ± 0.0005 while signals of Mn^{2+} ions were recorded with g-values of 2.00 ± 0.01 in leaves, shoots and fruits.

3.2.3. Determination of Soluble Sugar Content

Sugar contents (i.e. glucose, fructose, sucrose, and total sugar concentration) in dried leaf, shoot and fruit samples of the Kırmızı variety were determined according to the extraction method developed by Miron and Scahffer (1991) using HPLC (HP 1100 Series), RID (Refractive Index Detector) detector and Transgenomic CARBOSep COREGEL 87C (250 x 4.6 mm, 5µ) column in 3 repetitions. Distilled water used as mobile phase and HPLC conditions were 1.0 ml/min flow and 85°C oven temperature. Sugar contents in the samples were determined qualitatively and quantitatively using a refractive index detector according to the calibration curves created using the external standard and the retention time of the standard.

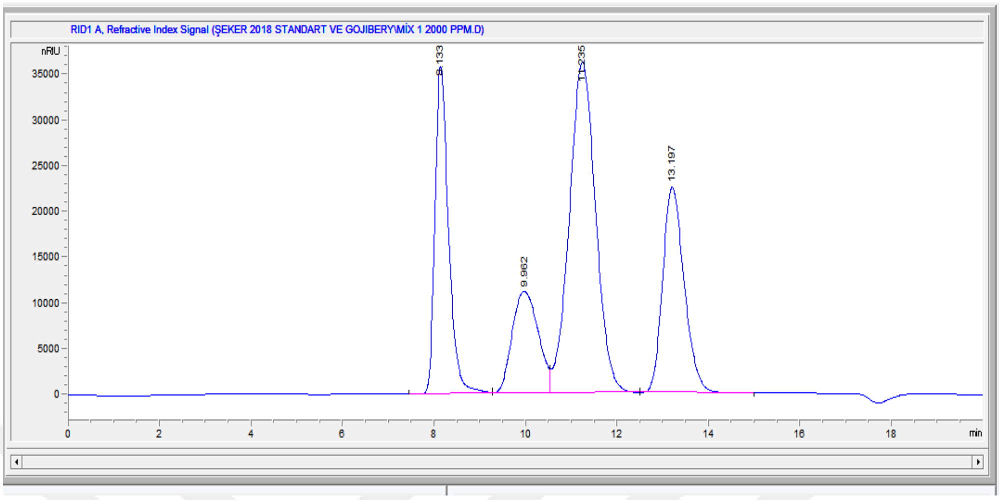

Figure 3.4. Chromatogram View of Sugar Analysis Standards.

3.2.4. Nitrogen Analysis

Nitrogen in leaf samples was determined by the 'Kjeldahl' method, which is a wet burning method recommended by Less (1951). Dried and homogenized samples were weighed 0.200 grams for each repetition and 1 Kjeldahl tablet and 12 ml of sulfuric acid were added to nitrogen combustion tubes and put into the incinerator. Samples were burned for a total of 1 hour: 10 minutes at 30 ^{o}C, 10 minutes at 60 ^{o}C and 40 minutes at 100 ^{o}C. The glass tube that complete the burning process was placed in the distillation device and the distillation process was started by placing 25 ml of boric acid in the flask into the other compartment. 0.1N HCl was added dropwise to our sample, which took the color of onion peel after distillation, and titrated until it turned into the color of boric acid before the reaction.

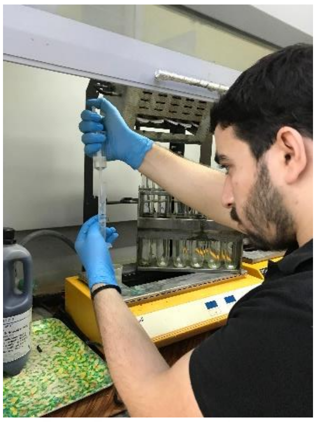

Figure 3.5. Burning of homogenized samples in incinerator

3.2.5. Phosphorus Analysis

The phosphorus content was determined by the method proposed by Barton (1948). 200 mg of dried and homogenized samples were taken and burned in as furnaces at 550 ^{o}C for 8 hours. The ash produced after burning was dissolved in 1/3 HCl and filtered in a blue band. The mixture prepared with 0.5µl Bartone solution and 4 ml of distilled water was vortexed. Vortexed samples were placed on plate and absorbance were measured in a spectrophotometer at a wavelength of 430 nanometers

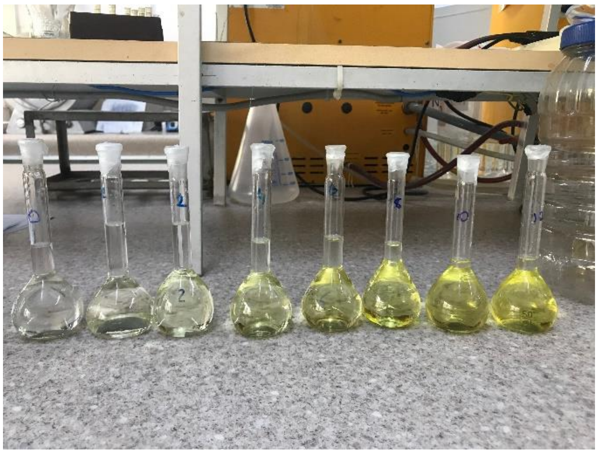

Figure 3.6. Preparation of standards for phosphorus analysis

3.2.6. Macro and Micro Nutrient Analysis

200 mg of dried and homogenized samples were taken and burned in an ashing furnace at 550 ^{o}C for 8 hours. The ash produced after the combustion was dissolved in 1/3 HCl and filtered in the blue band and the K, Ca, Mg, Fe, Zn, Mn and Cu concentrations were determined in the Varian brand Atomic Absorption Spectrometer (Chapman and Pratt, 1961).

4. RESULTS AND DISCUSSIONS

4.1. The effect of the flower cluster thinning application on the concentration of semiquinone radical and Mn^{2+} ions in organs of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

At the second term collection of plant material, electron paramagnetic resonance (EPR) analyzes showed significant increased generation semiquinone radicals in fruits and leaves of pistachio trees, where the flower cluster thinning application was made in relation to the control (i.e. without the flower cluster thinning application) (Figure 4.1 and 4.2).

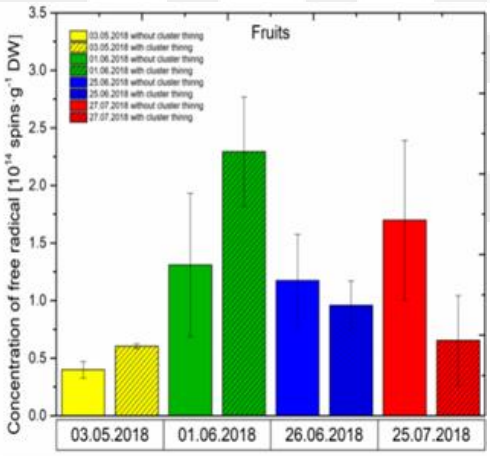

Figure 4.1. The concentration of semiquinone radical in fruits of pistachio trees (Pistacia vera L. cv. Kirmizi).

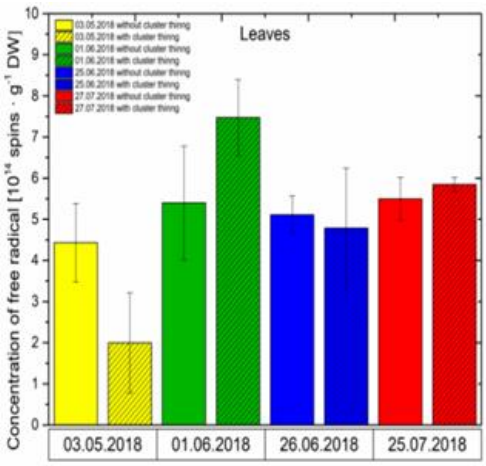

Figure 4.2. The concentration of semiquinone radical in leaves of pistachio trees (Pistacia vera L. cv. Kirmizi).

In turn, at the third period (26th June), in fruits and shoots, a significant decrease in the concentration of semiquinone radicals is visible, especially in shoots of trees with the flower cluster thinning. Moreover, the same trends have been seen in fruits and shoots in the recent period of collection of plant material, in case of the flower cluster thinning variant. Besides, the highest level of the mentioned radicals was in the shoots.

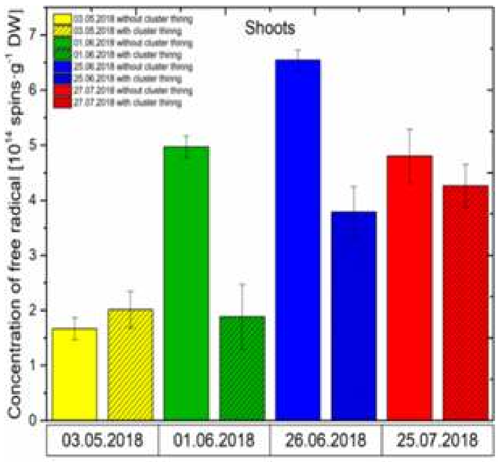

Figure 4.3. The concentration of semiquinone radical in shoots of pistachio trees (Pistacia vera L. cv. Kirmizi).

The concentration of manganese ions ($Mn^{2+)}$ showed fluctuations. The flower cluster thinning application caused a decrease in the level of manganese ions in leaves of pistachio trees in the first term (3rd May), while in shoots from this variant occurred increase these radicals in relation to the control (Figure 4.4). At the third period (26th June), in fruits and leaves were detected increases in concentrations of manganese ions (Figure 4.5, 4.6).The highest level of these radicals ($21x10^{14}$ Mn^{2+} ions g^{-1} DW) was found in leaves of pistachio as a result of the flower cluster thinning application.

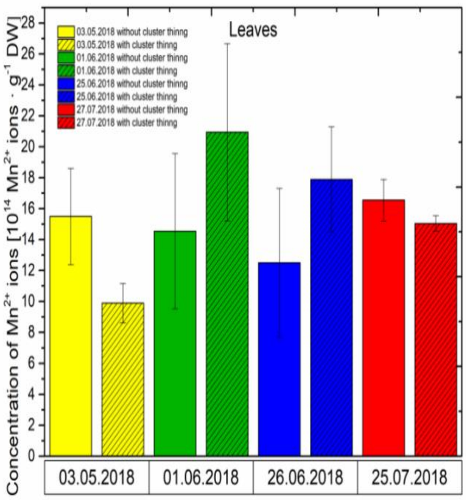

Figure 4.4. The concentration of manganese ions (Mn2+) in leaves of pistachio trees (Pistacia vera L. cv. Kirmizi).

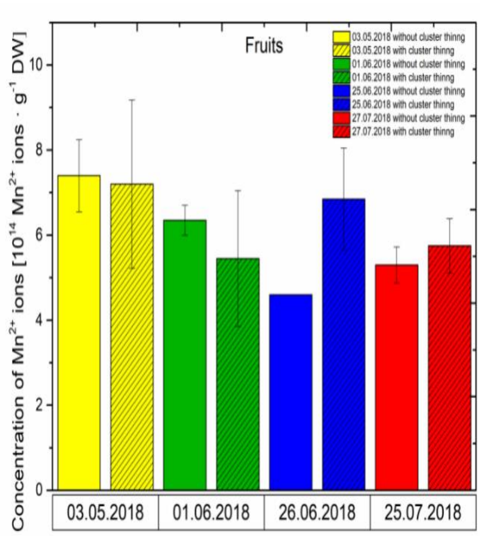

Figure 4.5. The concentration of manganese ions (Mn2+) in fruits of pistachio trees (Pistacia vera L. cv. Kirmizi).

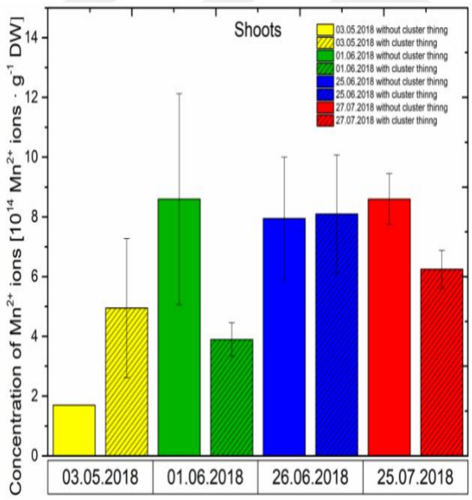

Figure 4.6. The concentration of manganese ions (Mn2+) in shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi).

4.2. The changes in the concentration of phytohormones after the of the flower cluster thinning application in pistachio organs (*Pistacia vera* L. cv. Kirmizi)

4.2.1. Abscisic acid (ABA) content

After the flower, cluster thinning application, the highest level of abscisic acid (ABA) was found in pistachio fruits (575 ng ABA· g^{-1}FW) at the first stage (in May) when nuts were collected (Figure 4.7).

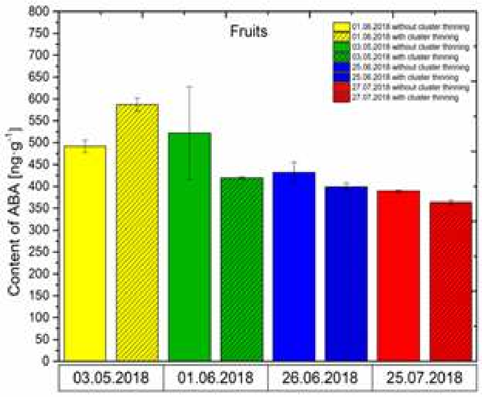

Figure 4.7. The concentration of abscisic acid (ABA) in fruits of pistachio trees (*Pistacia vera* L. cv. Kirmizi).

Also, a high ABA content was noted in pistachio shoots in the last period collecting samples in July (Figure 4.8).

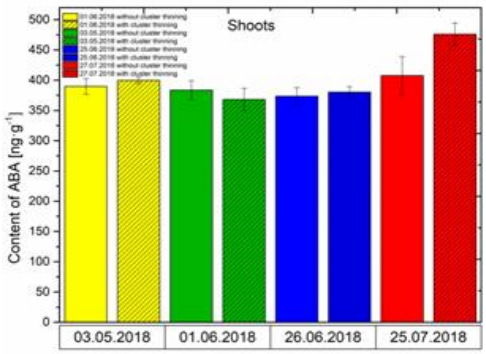

Figure 4.8. The concentration of abscisic acid (ABA) in shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi).

Attention is also drawn to the lower ABA level in fruits of pistachio trees from 1st June and leaves from 3rd May, where the flower cluster thinning application of pistachio trees was made (Figure 4.7 and 4.9).

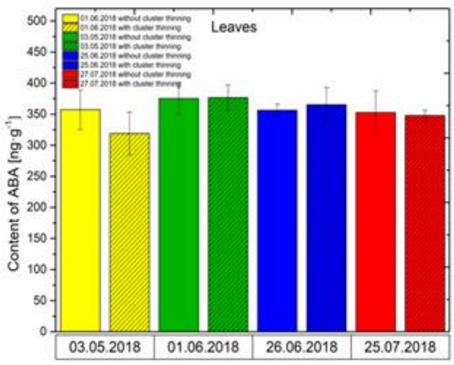

Figure 4.9. The concentration of abscisic acid (ABA) in leaves of pistachio trees (*Pistacia vera* L. cv. Kirmizi).

4.2.2. Salicylic acid (SA) content

It should be emphasized that after the flower cluster thinning application, the level of salicylic acid (SA) in the organs (fruits, leaves and shoots) was lower than in the control at certain time points when the samples were taken. For example, in June a significant reduction in the SA concentration in the fruits of pistachio trees when applied before sampling the flower cluster thinning in relation to the control was noted (Figure 4.10).

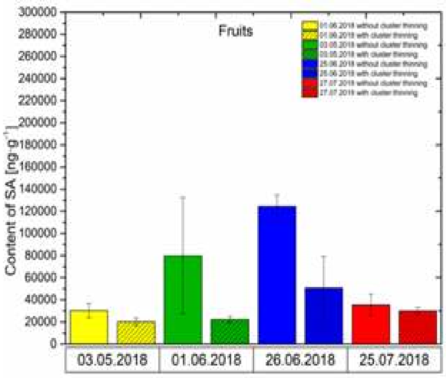

Figure 4.10. The concentration of salicylic acid (SA) in fruits of pistachio trees (*Pistacia vera* L. cv. Kirmizi).

In turn, in leaves, significant reduction in the SA level was also observed especially in samples from the first, third and fourth time of plant material collection. Besides, the highest level of SA was in fruits.

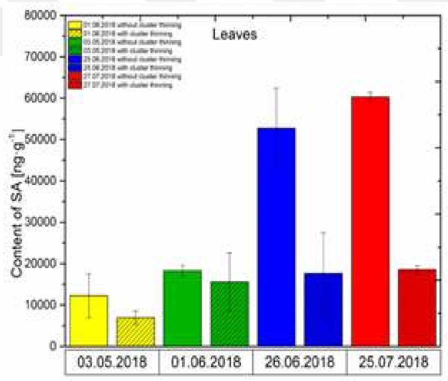

Figure 4.11. The concentration of salicylic acid (SA) in leaves of pistachio trees (*Pistacia vera* L. cv. Kirmizi).

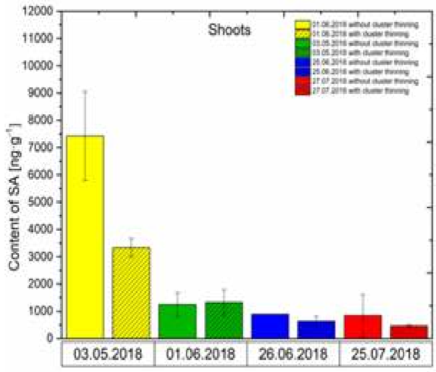

Figure 4.12. The concentration of salicylic acid (SA) in shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi).

At the first stage (in May), in fruits of pistachio trees where the flower cluster thinning application was made, indole-3-acetic acid (IAA) content was 4 times higher than in the control fruits. In turn, at the third time point in fruits of pistachio trees (where the flower cluster thinning application was done) 3 times lower concentration of IAA was found in relation to the control (Figure 4.13).

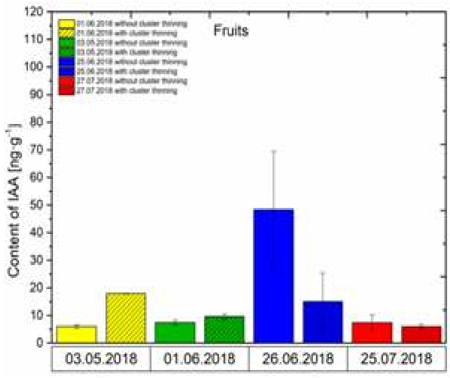

Figure 4.13. The concentration of indole-3-acetic acid (IAA) in fruits of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

A similar generally tendency in leaves and shoots at certain time points was noted. The highest level of IAA was found in the shoots of pistachio trees (Figure 4.15).

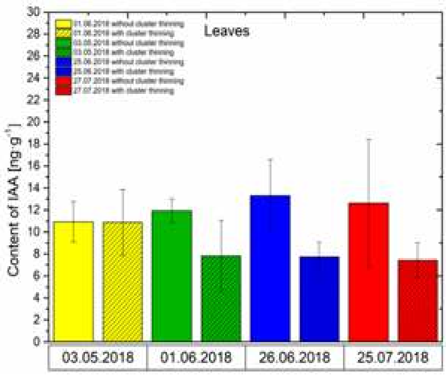

Figure 4.14. The concentration of indole-3-acetic acid (IAA) in leaves of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

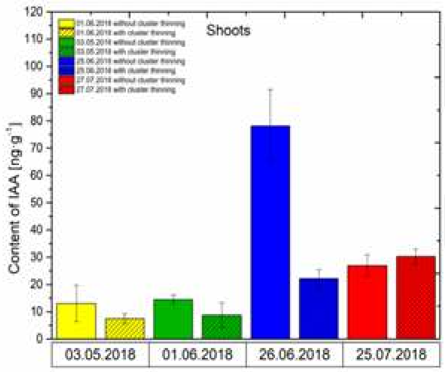

Figure 4.15. The concentration of indole-3-acetic acid (IAA) in shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

4.3. The changes in the level of sugars after the flower cluster thinning application in pistachio organs (*Pistacia vera* L. cv. Kirmizi)

In organs of pistachio (after the flower cluster thinning application), total sugar content was significantly higher than in the control. A similar tendency at the next time point (at 1st June) in fruits was observed (Figure 4.16).

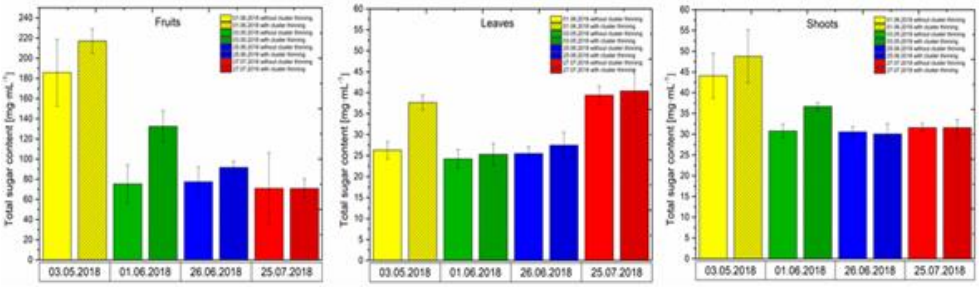

Figure 4.16. Total sugar content in fruits, leaves and shoots of pistachio (*Pistacia vera* L. cv. Kirmizi).

Besides, the highest It should be emphasized that a very high fructose content in the nuts of pistachio trees with the flower cluster thinning application was observed; it was 2.7 higher than in the control.

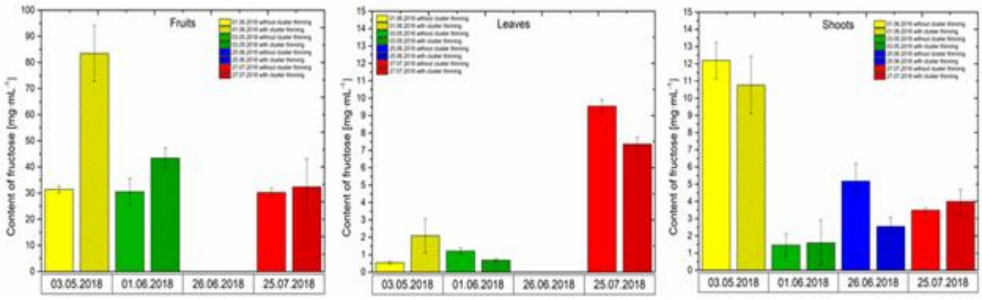

Figure 4.17. Fructose content in fruits, leaves and shoots of pistachio (*Pistacia vera* L. cv. Kirmizi).

Moreover, sucrose concentration in fruits of pistachio trees with the flower cluster thinning application collected in May was also higher than in the control; it was in these fruits 1.2 times higher than in the control.

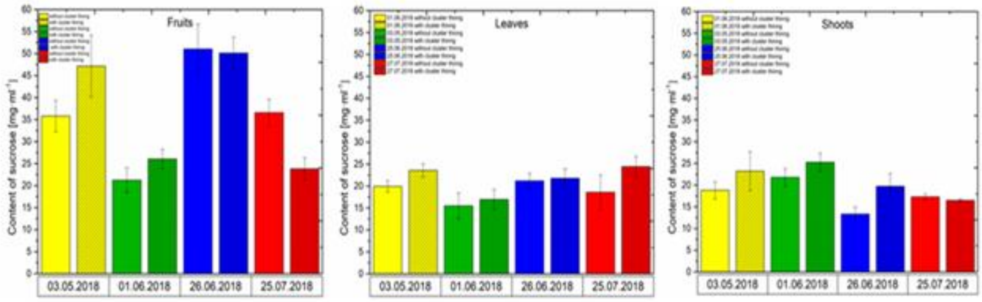

Figure 4.18. Sucrose content in fruits, leaves and shoots of pistachio (*Pistacia vera* L. cv. Kirmizi).

The content of monosaccharides (glucose and fructose) in fruits of pistachio trees with the flower cluster thinning application harvested in May was higher than in the control.

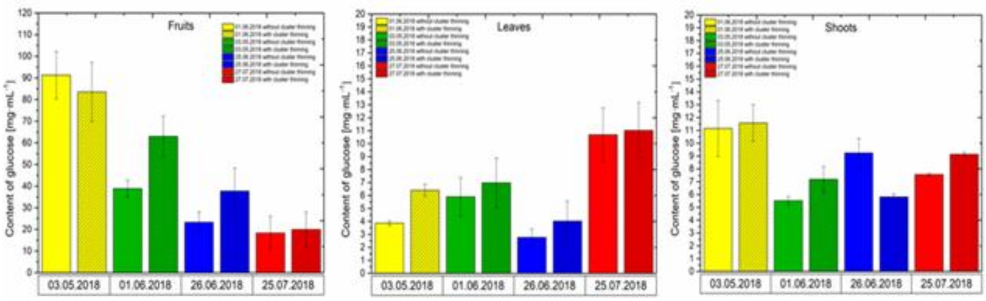

Figure 4.19. Glucose content in fruits, leaves and shoots of pistachio (*Pistacia vera* L. cv. Kirmizi).

4.4. The effect of the flower cluster thinning application on the concentration of mineral elements in pistachio organs (*Pistacia vera* L. cv. Kirmizi)

Analyzes of mineral elements in pistachio organs indicated that the Cu content in fruits was always higher after the flower cluster thinning application in relation to the control (without cluster thinning) (Figure 4.20).

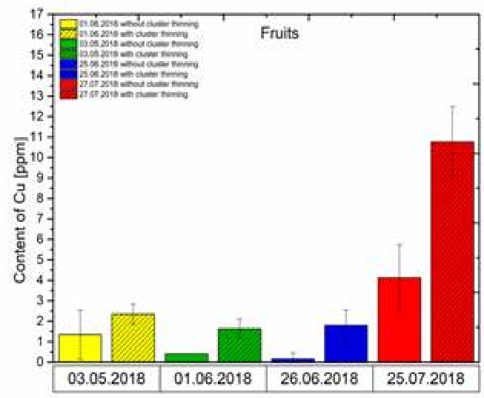

Figure 4.20. Cu contents in fruits of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

In leaves, the Cu content was higher on the second and third harvest dates in plants after the flower cluster thinning, while it was lower on the last sampling point in treated plants. In shoots, the content of Cu was higher on the second harvest point in plants after the flower cluster thinning, while it was lower on third and fourth harvest dates in treated plants in relation to the appropriate controls.

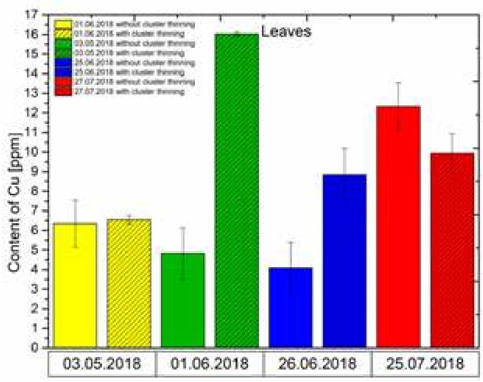

Figure 4.21. Cu contents in leaves of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

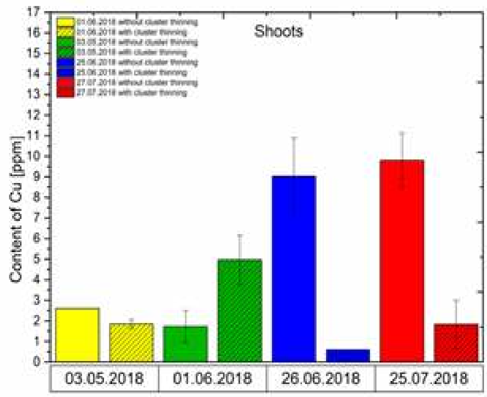

Figure 4.22. Cu contents in shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

The Mg content in fruits was always lower after the flower cluster thinning application in relation to the control (without cluster thinning). In leaves, the Mg content was lower on the first and second harvest dates in plants after the flower cluster thinning, while it was higher on the third sampling point in treated plants. In shoots, the most marked change is the lower Mg content of the treated plants on the last harvest date.

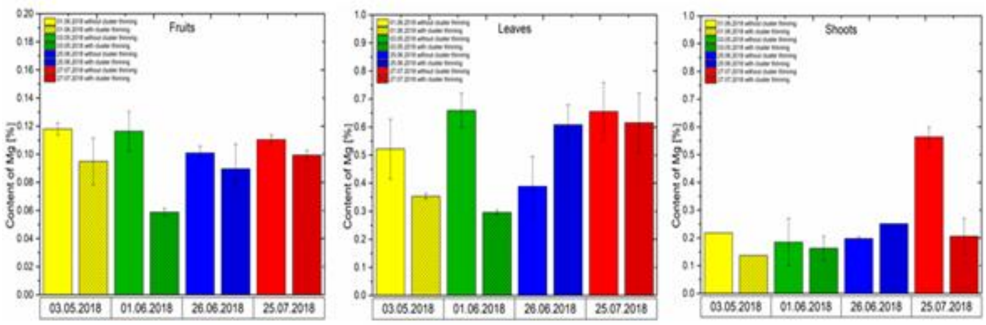

Figure 4.23. Mg contents in fruits, leaves and shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

In fruits, the Mn content was lower on the first date in plants after the flower cluster thinning, however, this content was higher on all other dates, in the treated plants compared to the untreated controls. Leaves sampled from treated plants presented lower levels of Mn on the first, second and fourth harvest dates, in shoots this trend continued and was noticeable in the second, third and fourth points of the plant material harvest.

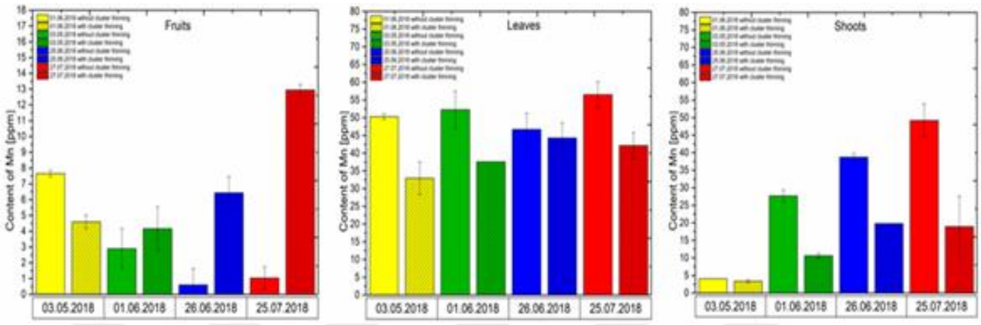

Figure 4.24. Mn contents in fruits, leaves and shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

In fruits, the N content was lower on the first date in plants after the flower cluster thinning, while it was higher on all other dates, in the treated plants compared to the untreated ones.

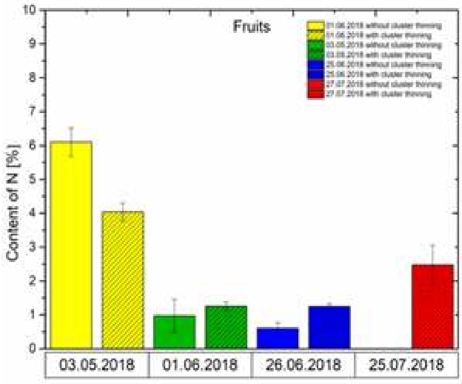

Figure 4.25. N contents in fruits of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

In leaves, the content of N was higher on the first, third and fourth harvest dates in treated plants in relation to the appropriate controls, however, there was less of it on the second harvest date in the treated plants than in control plants.

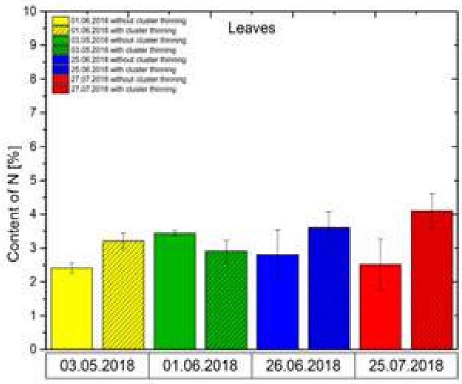

Figure 4.26. N contents in leaves of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

As for the content in the shoots, it was detected that the N level was lower on the second harvest date in plants after the flower cluster thinning, while it was higher on the third and fourth sampling points in treated plants.

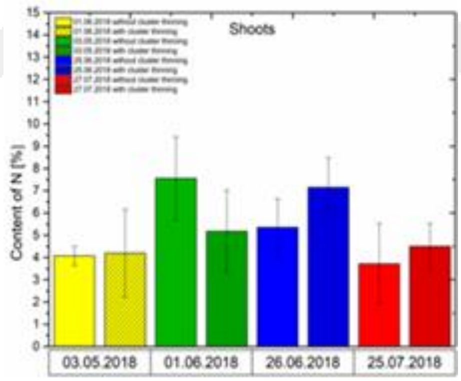

Figure 4.27. N contents in shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

Analyzes of the P content in pistachio organs revealed that its level in fruits was higher on the first, second and third harvest dates after the flower cluster thinning application in relation to the control and was lower on the last sampling point.

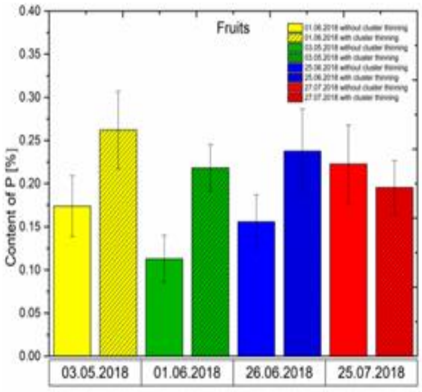

Figure 4.28. P contents in Fruits of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

In leaves, the P level was higher on the first, second and fourth harvest dates in treated plants in relation to the appropriate controls. Furthermore, the P content in shoots was higher on the first, second and third harvest dates in treated plants and lower on the last harvest date in treated ones.

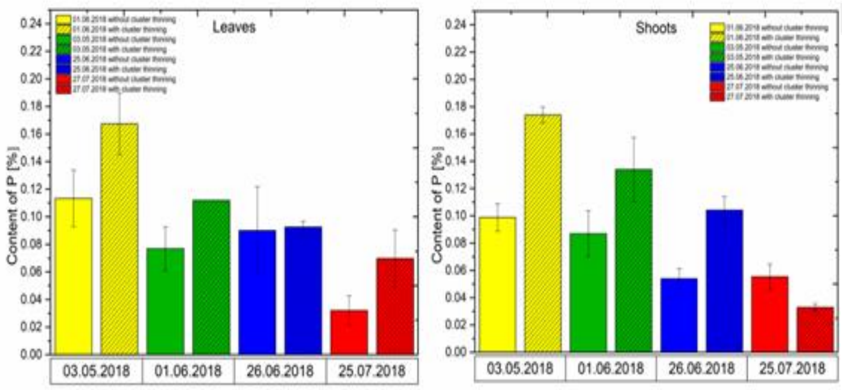

Figure 4.29. P contents in leaves and shoots of pistachio trees (*Pistacia vera* L. cv. Kirmizi)

Discussion: The results of this study for the first time show selected molecular and metabolic responses of *Pistacia vera* L.cv. Kirmizi overtime after the flower cluster thinning application. Firstly, within the first two terms collected fruits from pistachio trees with abscission of the flowers, semiquinone accumulation in relation to the control was demonstrated. Secondly, at first, term collected remarkably high accumulation of IAA was particularly observed in fruits of pistachio trees where the flower cluster thinning application was previously made. At this time point in these fruits, the ABA levels were also significantly higher than in the control.

The increase in the level of semiquinone radicals in pistachio fruits may be a consequence of a cluster thinning of the flowers or due to changes in the content of phenolic compounds during development and the plant cell redox status. Semiquinone radical formation was positively associated with two markers of oxidation (protein carbonyls and total peroxides) (Barbehenn et al., 2005). Nowadays, it is well known that semiquinone and phenoxyl radicals in plant cells are formed during the oxidation of hydroxyl groups in phenols and polyphenols (Barbehenn et al., 2003) and enzymes such as peroxidase (POX) or polyphenol oxidase are involved in these reactions (Kehrer 1993, Morkunas et al. 2013). Additionally, as previously demonstrated, semiquinone radicals are important in the defense mechanism of plants (Morkunas and Bednarski 2008; Mai et al., 2013; Woźniak et al., 2017; Morkunas et al., 2018). It is believed that these stable free radicals may be components of defense responses associated with the deposition of lignin-like polyphenolic materials, where they may perform a key function in restricting pathogen damage and spread (Hammel et al., 1986; Rayner and Boddy, 1988; Kalyanaraman, 1995). Research by Pearce et al., (1997) also suggested that semiquinones interact as free radical scavengers, quenching free radical chain reactions involved in the biodegradation of lignocelluloses, and thus protecting cell walls from decay and conferring a barrier function on the altered tissue. In the present study, EPR spectroscopy was applied to measure free radicals, including semiquinone radicals and paramagnetic metal ions (Mn^{2+}).

Moreover, the phytohormone levels (IAA, ABA, and SA) in organs at various stages of development were investigated. In present study, an increase in

IAA and ABA contents in fruits at the first harvest date were noted. A similar upward trend in IAA content during fruit set was also detected by Lee et al. (1997). The above authors also observed the increase in activity of soluble acid invertase that was accompanied an increase in endogenous IAA content. Auxin was found to affect any stage of fruit development. As reported Nitsch (1951), to produce flowers and fruits, plants have first to be shifted from a vegetative to a reproductive condition and this change is believed to be under hormonal control. It is well known that IAA, ABA, and SA are phytohormones involved in the signaling and regulation of many crucial processes in plants, including in fruit growth (Pérez-Llorca et al. 2019). Endogenous concentrations of IAA are particularly high at fruit set and during initial growth developmental stages (Pérez-Llorca et al. 2019 and references cited in this work). An interesting result seems to be the decline in SA levels in the organs of pistachio trees when applied before sampling the flower cluster thinning; SA contents in fruits, leaves and shoots were generally lower than in the control.

Additionally, sugar contents are important during pistachio fruit growth. As reported Zhang (2006), thinning a cluster prior to nut growth will result in a higher percentage of filled nuts on the thinned cluster. Therefore, it is suspected the tree's stored carbohydrate capacity initially determines the percentage of filled nuts. Production of blank nuts is strongly affected by alternate bearing. Probably, sugar status of the tree entering a crop year sets the limits on the crop load a tree can set and carry through maturation to splitting. Kazankaya et al. (2008) revealed that kernel sugar composition of nut crops changed based on species, variety, genotype, and accession was affected by different ecological conditions. The main sugar was sucrose in the majority of pistachio varieties, followed by glucose, fructose and maltose. In turn, in walnut genotypes, the main sugar was glucose in the majority and followed by sucrose, fructose and maltose. As for hazelnut, sucrose was the main sugar, followed by glucose, maltose, and fructose. This observation indicates a key role in the ripening of the fruit pistachio.

Pistachio trees are a biennial-bearing crop. Reducing carbohydrate cost in the "ON" year is important for overcoming biennial bearing. Before investigating ways to remove flower clusters in "ON" year, the distribution of flower cluster opening was studied. Two 18-years-old "Sirora" pistachio female trees were selected, and every flower cluster was tagged at full bloom with distinct markers for different dates. The number of open flower clusters were recorded, and distributions of flower cluster opening were also determined. This study has shown that although 2 trees have different numbers of flower buds, good flower buds emerged in the same period. Extra flower buds usually appeared at the later stages. This may be termed a homogeneous yield effect. Investigation also showed a tendency that late flower clusters usually have lower nut setting and produce small nuts with high percentages of blank nuts and non-split nuts.

5. CONCLUSION

There was an increase in the generation of semiquinone radicals (organic origin) in fruits of pistachio trees (*Pistacia vera* L. cv. Kirmizi) with the flower in relation to the control (without cluster thinning) at the first time point of harvesting the plant material.Significant accumulation of stable free radicals in pistachio tree leaves at the second sampling time point was also recorded.At selected time points at which the plant material was collected, a reduction in the level of semiquinone radicals in organs of pistachio trees with the flower cluster thinning application in relation to the control was noted.The concentration of paramagnetic metal ions (Mn^{2+}) demonstrated fluctuations.Extremely high accumulation of IAA in fruits of pistachio trees with flower cluster thinning has been demonstrated at first term collected.In pistachio fruits the highest level of ABA was found the first phase of collecting nuts.Higher level of ABA in fruits of pistachio trees with flower cluster thinning than in the control was found at the last time point of collecting nuts.Higher level of ABA in fruits of pistachio trees with flower cluster thinning than in the control was found at the last time point of collecting nuts.The significantly higher level of total sugars in the fruits of the pistachio trees with flower cluster thinning than in the control at the first time of nut harvest draws attention.Very high fructose content in the nuts of pistachio trees with the flower cluster thinning application was observed.Sucrose concentration in fruits of pistachio trees with the flower cluster thinning application collected at the first time was also higher than in the control.The content of monosaccharides (glucose and fructose) in fruits of pistachio trees with the flower cluster thinning application harvested at the first time was higher than in the control.Analyzes of mineral elements in pistachio organs indicated that the Cu content in fruits was always higher after the flower cluster thinning application in relation to the control.Phosphorus contents in organs pistachio trees with the flower cluster thinning application were higher than in the control, with the exception of phosphorus content in the organs that we collect at the last time point (in July).

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BIOGRAPHY

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