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Canan YILDIZ KUTLAR¹

Fatih ŞEN^{1*}

¹ Department of Horticulture, Faculty of Agriculture, Ege University, 35100 İzmir, Türkiye

* Corresponding author (Sorumlu yazar):

fatih.sen@ege.edu.tr

The effect of pre-harvest salicylic acid and gibberellic acid applications on-tree storability in 'Satsuma' mandarins (*Citrus unshiu* Marc.)

Satsuma mandarininde (*Citrus unshiu* Marc.) hasat öncesi salisilik asit ve giberellik asit uygulamalarının ağaçta depolamaya etkilerinin belirlenmesi

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ABSTRACT

Objective: Satsuma mandarin (*Citrus unshiu* Marc.), an important export product for Türkiye, is kept on the tree until the beginning of January. This study aimed to determine the effects of single and co-application of salicylic acid (SA) and gibberellic acid (GA₃) on the storability of Satsuma mandarin on trees.

Material and Methods: For this purpose, 20 ppm GA₃ two weeks before the color break period, 2 mM SA, and 1.5 ml/L Rezist at the color break period were applied alone and together to Satsuma mandarin fruit. Those without treatment were accepted controls. Various measurements and analyses were made in the samples taken at monthly intervals during storage on the tree.

Results: It was harvested in three periods at approximately one-month intervals from the beginning of November. The applications of SA or GA₃ alone or together limited the fruit drop rate during on-tree storage, the increase in the shell thickness, and the decrease in the specific gravity. Applications involving GA₃ delayed the coloration of fruits. The maturity index of the fruits treated with Rezist and GA₃ in the first harvest was lower.

Conclusion: The results showed that the pre-harvest applications of Satsuma mandarin would have positive contributions to the storage of fruits on the tree until the beginning of January by preventing fruit drop, and delaying the peel and fruit aging.

ÖZ

Amaç: Türkiye için önemli bir ihracat ürünü olan Satsuma mandarini ağaç üzerinde Ocak ayının başına kadar depolanmaktadır. Bu çalışmada, salisilik asit (SA) ve giberellik asitin (GA₃) teksel ve birlikte uygulanmasının Satsuma mandarinin ağaç üzerinde depolanabilirliğine etkilerinin belirlenmesi amaçlanmıştır.

Materyal ve Yöntem: Bu amaçla Satsuma mandarin meyvesine renk dönümünden iki hafta önce 20 ppm GA₃, renk dönümünde 2 mM SA ve 1.5 ml/L Rezist teksel ve birlikte uygulanmıştır. Uygulama yapılmayanlar kontrol olarak kabul edilmiştir. Kasım ayının başından itibaren aylık aralıklarla üç dönemde hasat edilmiştir.

Araştırma Bulguları: SA ve GA₃ uygulamalarının teksel ve birlikte yapılması ağaçta depolama süresince dökülen meyve oranını, kabuk kalınlığının artışı ve özgül ağırlığının azalışını sınırlandırmıştır. GA₃'ün yer aldığı uygulamalar meyvelerin renklenmesini geciktirmiştir. İlk hasatta Rezist ve GA₃ uygulanan meyvelerin olgunluk indisi daha düşük olmuştur.

Sonuç: Sonuçlar, Satsuma mandarin meyvelerine hasat öncesi yapılan uygulamaların meyve dökümünü engelleyerek, meyve ve kabuk yaşlanmasını geciktirerek ürünün ocak ayının başına kadar ağaç üstünde başarılı bir şekilde depolanabileceğini göstermiştir.

Keywords: Citrus fruits, fruit drop, fruit quality, on-tree storage, plant growth regulators

Anahtar sözcükler: Turunçgiller, meyve dökümü, meyve kalitesi, ağaçta depolama, büyüme düzenleyici maddeler

INTRODUCTION

Satsuma mandarin (*Citrus unshiu* Marc.) is a citrus fruit that is demanded and loved in the domestic and international markets. Mandarin has an important place in Türkiye's citrus production with 1.585.629 tons/year. Satsuma mandarin is an important foreign trade product for the Turkish economy, and its production is 767.482 tons/year (TÜİK, 2020).

In order to ensure the Satsuma mandarin to be introduced into the market for a longer time, it must be harvested late by being kept on the tree or stored in cold weather conditions. Although the harvest time of Satsuma mandarin in the Aegean Region varies more or less by year, it starts at the beginning of October. The harvest lasts until mid-January due to such reasons as the limited capacity of the factories processing and storing this product, the inability to have enough workers at the harvest, non-suitable climatic conditions for harvesting, and high price desire of producers. As the harvest continues, ripening continues in the fruits left on the tree, peel resistance disappears after a certain period, puffiness occurs, fruit drops increase, quality decreases due to aging, fruits become susceptible to diseases, and ultimately crop losses increase (Şen et al., 2013). In Satsuma mandarin, fruit drop is increasing due to various factors such as extended on-tree ripening time, inappropriate climatic conditions (precipitation, hoarfrost, wind, etc.), disease development, and insect damage. In some years, this drop rate can reach to 15-20% at the beginning of January (Şen et al., 2009). The fact that the fruits remaining on the tree undergo a rapid aging process also leads to a shortening of the period of taking Satsuma mandarins into cold storage. Because the losses increase with the lengthening of the storage period of the harvested fruits in cold storage at the next maturation stage (Mendilcioğlu, 1991).

In order to successfully store Satsuma mandarin stored on the tree, some plant growth regulators applied during the pre-harvest period ensure that ripening is delayed, product endurance is increased, and disease losses are reduced, which has a positive effect on prolonging the on-tree storage period (Ferguson et al., 1982; Davis, 1986). One of these plant growth regulators, salicylic acid (SA), affects a wide range of metabolic and physiological events, affecting the growth and development of plants. SA treatment has many effects such as decreased ethylene production, induction of disease resistance, prevention of oxidative stresses, induction of tolerance to cold damage, decrease in respiratory rate, decrease in ripening and aging speed, prevention of enzymes disrupting the cell wall and preservation of product hardness (Ding and Wang, 2003). Although the main role of SA is on biotic stresses, many studies show that SA also has important roles against several abiotic stresses such as cold stress, and heat shock (Ding et al., 2001; Ding and Wang, 2003). In recent years, it has been reported that commercial preparations containing Fe, Mn, and Zn together with salicylic acid stimulate the production of natural defense molecules against biotic stress by supporting and improving the natural defense system of the plant (Anonymous, 2020).

Gibberellic acid is another growth regulator substance in citrus fruits that is effective in the resistance of fruits during on-tree storage. It has been observed that the treatment of GA₃ before harvesting in many citrus species delays the aging, softening, deterioration, and coloration of the peel during the harvest period and reduces the loss of disease (El-Otmani & Coggins, 1991; Ismail & Wilhite, 1992; El-Otmani et al., 2000; Pozo et al., 2000; Tumminelli et al., 2005; Şen et al., 2009, 2013; Sezer et al., 2020). This, in turn, allows citrus fruits to be stored on the tree and prolongs the harvest period. For this purpose, it is recommended to apply GA₃, especially in orange (Tumminelli et al., 2005), lemon (El-Zeftawi, 1980), grapefruit (Ferguson et al., 1984), and mandarin (El-Otmani et al., 1990; Taminaga et al., 1998; Şen et al., 2009, 2013) varieties to be harvested in the late season.

Although there are studies on storing Satsuma mandarin on the tree using GA₃ treatment before harvesting, no previous study was found in which SA was applied together with GA₃ or alone. This study aims to determine the effects of single and co-administration of salicylic acid and gibberellic acid treatments on the on-tree storage of Satsuma mandarin.

MATERIALS and METHODS

Plant Material

The study was conducted on 'Owari Satsuma' mandarin trees grafted onto trifoliolate orange rootstock at commercial orchard located in Seferihisar, İzmir, western Türkiye (32°12'28.90"N, 26°49'09.29"E). The experimental orchard was established in 2006 with a planting distance of 4.5 m x 4.5 m, and irrigated via drip irrigation.

Salicylic acid has a purity of 99% (Merck, KGaA, China). Rezist™ is a commercial compound containing 1.75% Cu, 1.75% Mn, and 1.75% Zn (Stoller, USA). Gibberellic acid contains 1 g of the active substance in each tablet (Berelex, Hektaş, Türkiye).

20 ppm GA₃ was applied to Satsuma mandarin trees 2 weeks before the color break of the fruits, and SA 2 mM and Rezist 1.5 ml/L were applied on leaves at the color break period. GA₃ was also applied by combining SA (GA₃+SA) and Rezist (GA₃ + Rezist). During the first treatment period, only the trees where water was applied on leaves were accepted as the control. A pneumatic back sprayer was used to spray the foliage all over the tree (~ 5 L). Only water was sprayed on the control trees in the first treatment through a spreader-sticker (SPRAY-AIDE®, Miller Chemicals & Fertilizer, USA). Nitric acid was added to reduce the pH of the water used in the treatments to pH 6-7.

Harvest and Sampling

In the study related to the on-tree storage of Satsuma mandarin, the optimum harvest time was determined according to the maturation index (TSS/TA content) (Karaçalı, 2016). The first harvest was done when the WSDM/TA ratio was between 6.5-7, and since then, 3 more harvests were made at monthly intervals. The first harvest was done on October 17, 2019, at the optimum (normal) harvest time, while the other harvests were made on 12 November 2019 (1st Harvest), 9 December 2019 (2nd Harvest), and 4 January 2020 (3rd Harvest) respectively. During each harvest period, 30 samples of fruits (10 fruits from each tree) were taken from around the tree crown and from a height of 1.5 - 2 m from the ground. The study was planned as a Randomized Complete Block Design with 3 replications, and every 3 trees were accepted as a replication.

Fruit Drop and Quality Attribute

The weights of the fruits that dropped from the tree were determined during each harvest period, and the ratio (%) of the fruit drop was calculated by comparing this value to the total fruit weight of the tree. In addition, by examining the fruit drop, the rates of fruit drop due to ripening or decaying were also determined.

Twenty fruits selected to represent each replication were weighed with precision scales, and the average fruit weights, diameter, and length of these fruits were determined by a digital caliper, and peel thickness was determined by a micrometer.

The peel color was measured in CIE L*, a*, b* with a colorimeter (CR-400, Minolta Co., Tokyo, Japan) from both sides of the equatorial region of the fruits (McGuire, 1992). The specific gravity of the fruits was determined according to Karaçalı et al. (2001) in g/cm³. The juice yield was calculated by weighing the weight of the juice squeezed from the mandarins whose weights were determined (Şen, 2004).

After filtering the mandarin juice from the filter paper, the total soluble solids (TSS) content was determined by a refractometer (ATC-1, ATAGO, Italy), and the results were given as a percentage (%). The titratable acid (TA) content was detected with 5 ml of the sample taken from the juice, 0.1 N NaOH was dripped until the pH reached 8.1, which was measured with the help of a pH meter, thus the TA content was calculated in citric acid g/100 ml.

L-ascorbic acid (Vitamin C) content of the fruit juice was measured in the filtrate by using 2,6-dichloroindophenol according to the titrimetric method (AOAC, 1995). Absorbance at 518 nm was measured by spectrophotometer (Carry 100 Bio; Varian, Mulgrave, Australia). Total phenolic content was determined by the Folin-Ciocalteu method (Zheng & Wang, 2001). The absorbance was measured at 725 nm using a spectrophotometer and the results were expressed as mg gallic acid equivalent (GAE)/100 ml. The ferric reducing ability of plasma (FRAP) assay was performed as previously described by Benzie and Strain (1996), where reductants ("antioxidants") in the sample reduce Fe (III)/tripirydyltriazine complex to a blue ferrous form, with an increase in the absorbance at 593 nm. The final results are expressed in μmol trolox equivalents (TE)/ml, with reference to a trolox (25-500 $\mu\text{mol/l}$) standard curve.

Statistical Analyses

The data obtained from the experiment was subjected to analysis of variance using the statistical package program IBM® SPSS® Statistics 19 (IBM SPSS v19, NY, USA). For each on-tree storage, significant differences between the means for each year were determined by Duncan's multiple range tests at $p \leq 0.05$.

RESULTS

The effect of pre-harvest treatments on the proportion of fruits dropped during on-tree storage was statistically significant ($p \leq 0.05$) in the 3rd harvest, while it was insignificant in the 1st and 2nd harvests. During the 3rd harvest period, the fruit drop rate was found to be lowest (2.14%) in the trees treated with GA₃+Rezist and the highest (5.66%) in the control trees. In Rezist, SA, and GA₃ treatments, the rate of fruit drop was similar to each other. The rate of fruit drop in all treatments during the on-tree storage was found to be lower than the control. The effect of the treatments on fruit drop in the 1st and 2nd harvests was similar, ranging from 0.29% to 0.76% and 0.81% to 1.74%, respectively. With the increase in storage time on the tree, an increase in the rate of fruit drop was observed especially in the 3rd harvest period (Figure 1).

During the on-tree storage period, the effects of the treatments on ripening-induced fruit drops were significant only in the 3rd harvest ($p \leq 0.05$) (1.68-2.94%), and ripening-induced drops were lower in all treatments than the control (4.61%). The effect of the treatments on the fruit drop due to decaying was similar to each other, ranging from 0.43% to 1.04% (Figure 1).

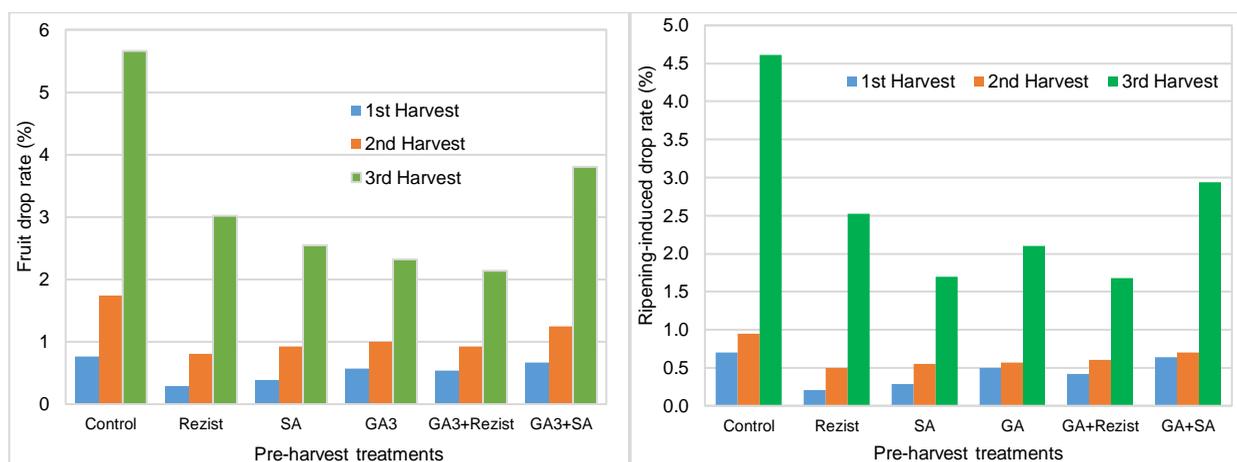


Figure 1. Effects of different pre-harvest treatments on the rate of fruit drop during on-tree storage and ripening-induced drop

Şekil 1. Hasat öncesi farklı uygulamaların ağaçta depolama süresince dökülen ve yaşlanma kaynaklı dökülen meyve oranına etkileri

The fruit weight and specific weight of Satsuma mandarin fruits during the on-tree storage period are given in Table 1 based on pre-harvest treatments. The effect of different pre-harvest treatments on fruit weight during on-tree storage was similar to each other, with fruit weights ranging between 99.64 g - 111.92 g, 100.78 g - 115.55 g, and 110.09 g - 120.05 g in the 1st, 2nd, and 3rd harvest, respectively.

The effect of the treatments on the specific weight of mandarin fruit during the on-tree storage period differed significantly, and the specific weights of the fruits in the treatments with GA₃ were found to be 7.7%, 12.3%, and 16.2% higher in the 1st, 2nd and 3rd harvests, respectively, compared to the control. At the end of the 3-month on-tree storage period, the specific weight of the fruits was between 0.88-89 g/cm³ in the treatments with GA₃ and 0.76 g/cm³ in the control.

Table 1. Effects of different pre-harvest treatments on the fruit weight and specific weight of Satsuma mandarin

Çizelge 1. Hasat öncesi farklı uygulamaların ağaçta depolama süresince Satsuma mandarinin meyve ağırlığı ve özgül ağırlığına etkileri

Treatments	Fruit weight (g)			Specific weight (g/cm ³)		
	1 st Harvest	2 nd Harvest	3 rd Harvest	1 st Harvest	2 nd Harvest	3 rd Harvest
Control	108.28±7.58 ^{NS}	116.08±6.34 ^{NS}	118.69±4.74 ^{NS}	0.87±0.02 ^{b z*}	0.81±0.03 ^{b**}	0.76±0.07 ^{b*}
Rezist	103.25±9.96	103.49±4.20	115.09±9.65	0.90±0.02 ^{ab}	0.88±0.05 ^a	0.86±0.02 ^{ab}
SA	99.64±4.90	100.78±6.27	110.09±4.58	0.91±0.04 ^{ab}	0.89±0.03 ^a	0.80±0.10 ^{ab}
GA ₃	111.92±8.78	115.55±6.07	120.05±8.71	0.93±0.03 ^a	0.91±0.01 ^a	0.88±0.02 ^a
GA ₃ +Rezist	101.96±9.81	104.72±7.80	112.38±9.58	0.95±0.01 ^a	0.92±0.02 ^a	0.89±0.02 ^a
GA ₃ +SA	102.00±9.02	108.73±3.68	114.36±4.56	0.93±0.03 ^a	0.90±0.02 ^a	0.88±0.01 ^a

^z Mean separation within columns by Duncan's multiple range test, $p \leq 0.05$.

^{NS}, *, **, Non-significant or significant at $p \leq 0.05$, or 0.01, respectively

Changes in the diameter, and length of Satsuma mandarin during on-tree storage according to the treatments are presented in Table 2. The effect of treatments on fruit diameter was significant in the 3rd harvest ($p \leq 0.05$) and the diameter of control fruits was higher than those treated with GA₃ + Resist and GA₃ + SA. The effect of different pre-harvest treatments on fruit length differed significantly in the 3rd harvest ($p \leq 0.05$), while fruit length was the highest with 52.26 mm in GA₃ and lowest with 47.35 mm in GA₃ + Resist treatment.

Table 2. Effects of different pre-harvest treatments on the diameter, and length of Satsuma mandarin

Çizelge 2. Hasat öncesi farklı uygulamaların ağaçta depolama süresince Satsuma mandarinin eni ve boyuna etkileri

Treatments	Fruit diameter (mm)			Fruit length (mm)		
	1 st Harvest	2 nd Harvest	3 rd Harvest	1 st Harvest	2 nd Harvest	3 rd Harvest
Control	64.40±1.98 ^{NS}	65.20±1.01 ^{NS}	69.70±0.77 ^{a z*}	47.80±2.06 ^{NS}	49.90±0.78 ^{NS}	50.96±1.54 ^{ab*}
Rezist	63.80±2.40	63.17±0.87	67.77±2.65 ^{ab}	50.10±2.76	48.32±1.85	51.03±1.20 ^{ab}
SA	62.00±1.15	63.03±1.51	66.55±1.85 ^{ab}	49.80±0.78	46.71±1.53	48.82±0.68 ^{bc}
GA ₃	64.20±3.43	65.10±1.85	67.63±2.36 ^{ab}	51.50±2.23	47.62±1.44	52.26±2.83 ^a
GA ₃ +Rezist	63.40±3.00	64.21±2.67	65.03±2.19 ^b	50.10±1.77	47.55±1.54	47.35±0.92 ^c
GA ₃ +SA	62.50±2.41	64.83±1.80	65.44±0.81 ^b	48.90±2.04	47.62±1.25	48.00±1.24 ^{bc}

^z Mean separation within columns by Duncan's multiple range test, $p \leq 0.05$.

^{NS}, *, Non-significant or significant at $p \leq 0.05$, respectively.

The peel a* and b* values of mandarin fruits formed by different pre-harvest treatments during on-tree storage are given in Figure 2. The effect of treatments to the value a* (+ represents red, - represents green on the horizontal axis) of Mandarin peels differed significantly during all harvest periods. The a*

values of mandarin peels in the groups with GA₃ during on-tree storage were found to be lower than those of control, SA, and Rezist treatments. While this difference was very pronounced in the first harvest, it decreased in the later harvest periods. The a* value of the fruit peels was found to be 135%, 15%, and 10% lower in the 1st, 2nd, and 3rd harvests, respectively, compared to GA₃ treatments. The effect of different treatments on the b* value of mandarin peel was found to be significant in the 1st and 2nd harvests during on-tree storage, and in general, the peel b* value (+ represents yellow and - represents blue on the vertical axis) was found to be lower in the treatments with GA₃ compared to the others.

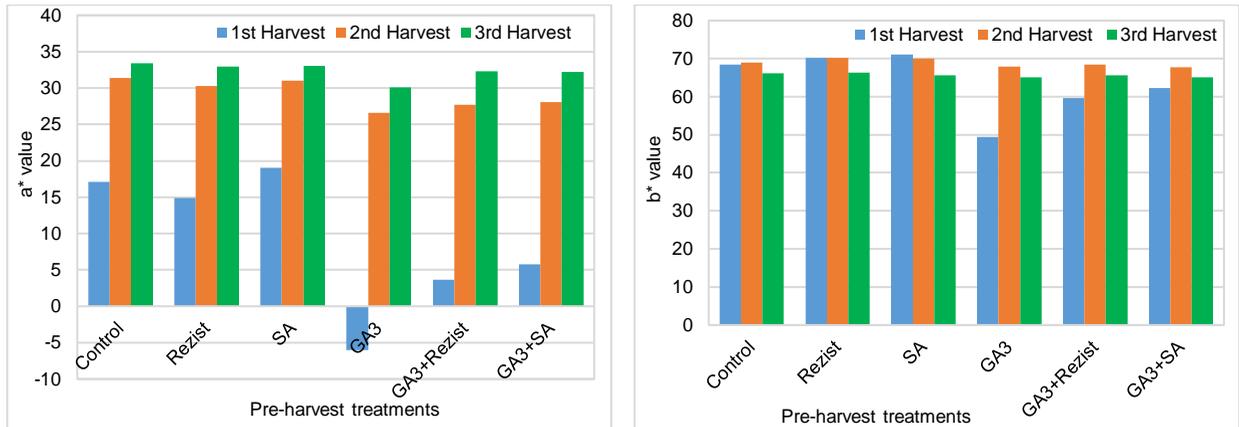


Figure 2. Effects of different pre-harvest treatments on the peel a* and b* values during on-tree storage

Şekil 2. Hasat öncesi farklı uygulamaların ağaçta depolama süresince Satsuma mandarinin kabuk a* ve b* değerine etkileri

The peel thickness, and fruit juice ratio of Satsuma mandarin fruits during the on-tree storage period are given in Table 3 based on pre-harvest treatments. The effect of treatments on the peel thickness of Mandarin fruit differed significantly during the on-tree storage period ($p \leq 0.05$), while the peel thickness of the control fruits was found to be the highest, SA treatment was the lowest in the 1st harvest, and among the lowest treatments in the 2nd and 3rd harvests.

The effect of treatments on the juice ratio was significant in the 1st and 3rd harvest ($p \leq 0.01$), the juice ratio of the GA₃ group in the 1st harvest and all treatment groups in the 3rd harvest were found to be higher than the control. At the end of the on-tree storage period, the juice ratio of the treated mandarins was 24% higher on average than the control.

Table 3. Effects of different pre-harvest treatments on the peel thickness, and fruit juice ratio of Satsuma mandarin fruits during on-tree storage

Çizelge 3. Hasat öncesi farklı uygulamaların ağaçta depolama süresince Satsuma mandarinin kabuk kalınlığı ve meyve suyu oranına etkileri

Tretments	Peel thickness (mm)			Fruit juice ratio (%)		
	1 st Harvest	2 nd Harvest	3 rd Harvest	1 st Harvest	2 nd Harvest	3 rd Harvest
Control	2.28±0.49 ^{a z}	2.66±0.13 ^{a*}	3.19±0.17 ^{a*}	47.17±3.58 ^{b**}	48.56±4.87 ^{NS}	39.77±1.47 ^{d**}
Rezist	1.99±0.13 ^{ab}	2.35±0.13 ^{ab}	2.71±0.39 ^b	47.69±1.80 ^b	45.82±2.36	46.37±3.08 ^c
SA	1.73±0.14 ^b	2.24±0.17 ^b	2.58±0.24 ^b	47.90±2.16 ^{ab}	46.43±1.17	50.11±2.99 ^{abc}
GA ₃	2.17±0.11 ^a	2.34±0.20 ^{ab}	2.66±0.25 ^b	52.79±1.88 ^a	50.77±1.48	50.97±2.17 ^{ab}
GA ₃ +Rezist	2.06±0.26 ^{ab}	2.11±0.08 ^b	2.64±0.22 ^b	51.49±4.54 ^{ab}	47.41±4.05	47.20±4.37 ^{bc}
GA ₃ +SA	2.13±0.10 ^{ab}	2.33±0.35 ^{ab}	2.75±0.11 ^{ab}	50.54±3.69 ^{ab}	49.37±2.76	51.88±1.25 ^a

^z Mean separation within columns by Duncan's multiple range test, $p \leq 0.05$.

^{NS}, *, **, Non-significant or significant at $p \leq 0.05$, or 0.01, respectively

The TSS, and TA content of mandarin fruits during the on-tree storage period is presented in Table 4. The effect of treatments on the TSS content during the on-tree storage period was significant in harvest 3 ($p \leq 0.05$) and the TSS content was found to be higher in GA₃ + SA treatments than in control and Rezist treatments.

The effect of different pre-harvest treatments on the TA content of fruits differed significantly during the on-tree storage period ($p \leq 0.05$). Although the effect of the treatments on the TA content did not change decisively, the TA content of mandarins treated with GA₃+SA and Rezist was generally higher than in SA and control groups.

Table 4. Effects of different pre-harvest treatments on the TSS, and TA content of Satsuma mandarin fruits during on-tree storage

Çizelge 4. Hasat öncesi farklı uygulamaların ağaçta depolama süresince Satsuma mandarinin SÇKM ve TA miktarına etkileri

Tretments	TSS content (%)			TA content (g/100 ml)		
	1 st Harvest	2 nd Harvest	3 rd Harvest	1 st Harvest	2 nd Harvest	3 rd Harvest
Control	11.83±0.40 ^{NS}	12.83±0.83 ^{NS}	11.70±0.30 ^{b z'}	1.03±0.07 ^{bc'}	0.83±0.05 ^{b'}	0.81±0.05 ^{b'}
Rezist	11.13±0.57	12.30±0.98	11.97±0.21 ^b	1.17±0.09 ^a	0.92±0.10 ^a	0.84±0.02 ^{ab}
SA	12.03±0.99	12.87±0.93	12.53±0.58 ^{ab}	0.97±0.07 ^c	0.82±0.09 ^b	0.81±0.03 ^b
GA ₃	11.10±0.25	11.97±1.22	12.47±0.84 ^{ab}	1.13±1.13 ^{ab}	0.84±0.04 ^{ab}	0.81±0.02 ^b
GA ₃ +Rezist	11.57±0.70	12.27±0.25	12.23±0.61 ^{ab}	1.12±1.14 ^{ab}	0.91±0.03 ^{ab}	0.88±0.04 ^{ab}

^z Mean separation within columns by Duncan's multiple range test, $p \leq 0.05$.

^{NS}, *, Non-significant or significant at $p \leq 0.05$, respectively.

The effects of different pre-harvest treatments on the maturation index, and vitamin C of Satsuma mandarin during on-tree storage are given in Table 5. The effect of pre-harvesting treatments on the maturation index of mandarin fruit was significant in the 1st harvest ($p \leq 0.05$), and the maturation index of mandarins treated with Rezist and GA₃ was lower than the control and other treatments.

The effects of different pre-harvest treatments on vitamin C, total phenol content, and antioxidant activity of Satsuma mandarin during on-tree storage are given in Table 4. The effect of the treatments on the vitamin C content in satsuma mandarin during on-tree storage was similar, ranging from 21.45 mg/100 ml to 27.48 mg/100 ml.

Table 5. Effects of different pre-harvest treatments on the maturation index, and vitamin C of Satsuma mandarin fruits during on-tree storage

Çizelge 5. Hasat öncesi farklı uygulamaların ağaçta depolama süresince Satsuma mandarinin olgunluk indisi ve C vitamini miktarına etkileri

Tretments	Maturation index			Vitamin C (mg/100 ml)		
	1 st Harvest	2 nd Harvest	3 rd Harvest	1 st Harvest	2 nd Harvest	3 rd Harvest
Control	11.54±1.11 ^{ab z'}	15.50±0.72 ^{NS}	14.51±0.60 ^{NS}	22.47±2.71 ^{NS}	25.76±2.02 ^{NS}	21.45±2.06 ^{NS}
Rezist	9.56±1.07 ^c	13.43±0.78	14.32±0.50	26.48±1.95	27.48±1.96	22.61±1.92
SA	12.43±0.74 ^a	15.74±1.32	15.44±0.94	24.11±2.22	25.66±2.42	22.13±2.59
GA ₃	9.81±0.49 ^c	14.30±1.08	15.60±1.05	23.68±2.01	25.89±1.70	24.11±2.58
GA ₃ +Rezist	10.31±1.70 ^{bc}	13.49±0.35	13.87±0.74	25.18±1.74	25.93±1.81	25.62±2.73
GA ₃ +SA	10.60±0.28 ^{bc}	13.56±0.89	14.64±1.35	24.22±2.90	26.06±0.92	23.34±1.61

^z Mean separation within columns by Duncan's multiple range test, $p \leq 0.05$.

^{NS}, *, Non-significant or significant at $p \leq 0.05$, respectively.

The effect of the treatments on the total phenol content of satsuma mandarin was insignificant, the total phenol content ranging between 44.45-51.54 mg GAE/100 ml in the 1st harvest ranged from 40.45 to 47.33 mg GAE/100 ml in 3rd harvest. The antioxidant activity of the treatments during on-tree storage was similar, ranging from 4.17 to 5.91 µmol TE/ml (Table 6).

Table 6. Effects of different pre-harvest treatments on the total phenol content, and antioxidant activity of Satsuma mandarin fruits during on-tree storage

Çizelge 6. Hasat öncesi farklı uygulamaların ağaçta depolama süresince Satsuma mandarinin toplam fenol miktarı ve antioksidan aktivitesine etkileri

Treatments	Total phenol content (mg GAE/100 ml)			Antioxidant activity (µmol TE/ml)		
	1 st Harvest	2 nd Harvest	3 rd Harvest	1 st Harvest	2 nd Harvest	3 rd Harvest
Control	45.32±4.43 ^{NS}	43.80±5.85 ^{NS}	40.81±1.39 ^{NS}	5.78±0.60 ^{NS}	4.88±0.44 ^{NS}	4.39±0.12 ^{NS}
Rezist	49.97±1.58	42.76±2.65	40.45±3.26	5.54±0.21	5.13±0.54	4.17±0.42
SA	51.54±1.70	42.69±4.95	42.91±1.25	5.91±0.65	4.74±0.33	4.29±0.28
GA ₃	48.80±4.98	46.14±1.85	42.38±4.20	5.67±0.28	4.79±0.22	4.51±0.30
GA ₃ +Rezist	50.06±4.44	47.33±4.78	42.61±3.69	5.85±0.14	5.19±0.32	4.52±0.51
GA ₃ +SA	44.45±1.95	40.81±2.42	43.76±2.27	5.46±0.23	4.59±0.26	4.36±0.17

^{NS}, Non-significant.

DISCUSSION and CONCLUSION

In order to introduce Satsuma mandarins to the market for a longer period of time, the on-tree storage, as well as storage in cold weather conditions, must be fulfilled successfully. Delaying the ripening and aging of fruits on the tree will contribute to the success of on-tree storage. The treatments limit the rate of fruit drop during on-tree storage compared to the control, which is because salicylic acid and gibberellic acid inhibit ethylene biosynthesis in fruits, delaying aging. These treatments limit the effect of ethylene, slowing aging, maintaining peel resistance, and delaying the fruit to break from the stem. Therefore, it is thought that SA and GA₃ are effective in slowing down fruit aging and maintaining peel resistance, and reducing fruit drops. SA and GA₃ delayed aging by inhibiting ethylene synthesis, this result is compatible with previous studies in mandarin (Ritenour et al., 2005), banana (Srivastava and Dwivedi, 2000), apple, and pear (Romani et al., 1989; Babalar et al., 2007) fruits.

The treatments did not affect fruit weight, diameter, and length or showed instability. This is because the treatments were made during and after the optimum harvest period, during which the development was largely completed (Şen et al., 2013).

The delaying effect of the treatments with GA₃ on the coloring of fruits depends on the fact that it slows down the degradation of chlorophyll in fruits treated with GA₃. This effect of GA₃ decreased with the progression of on-tree storage duration. One of the most obvious changes that occur during the ripening of mandarin fruit is the degradation of chlorophyll, which gives the peel a green color, and this is replaced by yellow-red carotenoids, and coloration occurs (Senthilkumar & Vijayakumar, 2014). The effect of GA₃ treatment on delaying coloration by slowing down the loss of chlorophyll in citrus fruits has been revealed in many studies (Şen et al., 2013; Rokaya et al., 2016; Sezer et al., 2020).

A more significant decrease in the value of the specific gravity of control fruits with the progression of the on-tree storage period indicates that the peel resistance of fruits decreases, the albedo texture of fruits deteriorates, and the tendency to puffiness increases. Puffiness is the separation of the softened peel from the fruit flesh, which is observed with the progression of the ripening of mandarin fruits (Karaçalı, 2016). The specific gravity of the treated fruits is higher than the specific gravity of the control

fruits, which can be explained by the delaying effect of the treatments on the ripening of the fruits. This delays the formation of puffiness, which is an aging disorder, when on the tree.

The low peel thickness of the treated mandarin fruits can be explained by the fact that SA, GA₃, and Rezi treatments limit the increase in peel thickness and slow down the deterioration of the peel during the on-tree storage of fruits. The thin peel of Satsuma mandarin fruits is an indicator of their quality and strength in storage, while the thickening of the peel is an indicator of the increase in the fruit's sensitivity to puffiness and a decrease in quality. Indeed, the effect of treatments limiting the increase in peel thickness is compatible with the fact that the specific gravity values of the treated fruits are higher than the control. The treatment of GA₃ reduced the peel thickness in the mandarin (Garcia-Luis et al., 1992; Pozo et al., 2000) and Valencia Navel orange (Sezer et al., 2020) varieties.

The higher juice yield in the treated mandarins than in the control and the thickening of the peel at the last harvest may be associated with increased puffiness. As the tendency to puffiness increases, the juice ratio decreases (Karaçalı, 2016).

The effect of pre-harvest treatments was limited and/or unstable on the TSS and TA content of the Satsuma mandarin during on-tree storage, indicating that there was no significant effect. It is believed that the differences are caused not by treatment, but by sampling. Also, it was observed that GA₃ treatment did not have a significant effect on the TSS content in orange, mandarin, and grapefruit stored on the tree (Ben-İsmail et al., 1995; Ritenour et al., 2005; Balkıç et al., 2019). The low maturation index of mandarin fruits treated with Rezi and GA₃ in the 1st harvest can be explained by the aging-delaying effect of the treatments. Because the low TSS content and the high TA content in these treatments are compatible with slow aging.

The effect of the treatments on vitamin C, total phenol content, and antioxidant activity during on-tree storage was limited, and we thought that this depended on the fact that the treatments were made during the optimum harvest and post-harvest periods. It is reported that the treatment of GA₃ during these periods specified before harvesting has no or very little effect on the biochemical properties of many citrus fruits (Coggins, 1982; Pozo et al., 2000). Studies are showing that salicylic acid treatments increase the total amount of phenol and antioxidant activity in fruits and vegetables (Huang et al., 2008; Tareen et al., 2012; Aghdam et al., 2016; Haider et al., 2021), but the time of treatment in these studies varies. Therefore, it is thought that the time of treatment is effective in limiting the effect of treatments on the biochemical properties of the fruit.

The pre-harvest treatments limited the rate of fruit drop during on-tree storage, the increase in peel thickness, and the decrease in specific gravity compared to the control. Treatments with GA₃ delayed the coloring of fruits. In general, the juice yield was found to be higher in the treated mandarins than in the control. The maturation index of fruits treated with Rezi and GA₃ at the first harvest was lower. The effects of the treatments on TSS, TA, total phenol content, vitamin C, antioxidant activity, fruit weight, diameter, and length of fruits during on-tree storage were limited and/or showed instability.

Considering all the data, the treatment of GA₃ two weeks before the color break and the treatments of SA and Rezi at the color break prevented fruit drop and delayed the peel and fruit aging, and contributed positively to the on-tree storage of the Satsuma mandarins until the beginning of January.

Data Availability

Data will be made available upon reasonable request.

Author Contributions*

Conception and design of the study: CYK, FŞ; sample collection: CYK; analysis and interpretation of data: CYK; statistical analysis: FŞ; visualization: FŞ; writing manuscript: CYK, FŞ.

Conflict of Interest

There is no conflict of interest between the authors in this study.

Ethical Statement

We declare that there is no need for an ethics committee for this research.

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