International Journal of Agriculture, Forestry and Life Sciences 2026; 10 (1): 9-20 Research Article
https://doi.org/10.66598/ijafls.1901754 https://izlik.org/JA63MF35KD

THE EFFECT OF YEAST (*Saccharomyces cerevisiae*) APPLICATION ON FRUIT QUALITY IN APPLES

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Received: March 3, 2026 | **Accepted:** April 16, 2026 | **Published Online:** April 29, 2026

ABSTRACT
The increasing demand for sustainable and environmentally friendly production practices has intensified interest in microbial-based inputs as alternatives to synthetic agrochemicals in fruit cultivation. In this study, the effects of different doses of *Saccharomyces cerevisiae* on yield, pomological characteristics, physicochemical quality parameters, and antioxidant properties were investigated in two apple cultivars, *Summer Red* and *Mondial Gala*. The experiment was conducted during the 2024–2025 growing season under the ecological conditions of Eskişehir, Türkiye, using a randomized block design with four application doses (0, 5, 10, and 15 ppm). Results indicated that the response to *S. cerevisiae* applications was strongly dependent on cultivar and dose. In *Summer Red*, low-dose application (5ppm) significantly increased yield, total phenolic content, and ascorbic acid concentration, while higher doses showed limited or inconsistent effects on fruit quality traits. In contrast, *Mondial Gala* exhibited greater sensitivity to the treatments, with moderate doses (5 and 10 ppm) enhancing fruit weight and width, whereas higher doses tended to suppress certain quality attributes, particularly flavonoid and ascorbic acid contents. Color parameters and fruit firmness were generally less affected by the applications in both cultivars. Multivariate analyses (PCA and correlation analysis) revealed a clear separation between cultivar–dose combinations, highlighting distinct relationships between yield, fruit quality, and antioxidant parameters. Overall, the findings demonstrate that *S. cerevisiae* can be considered a promising biostimulant in apple cultivation; however, its effectiveness is highly genotype-specific and dose-dependent. Determination of optimal application doses tailored to individual cultivars is therefore essential to maximize both yield and fruit quality under sustainable production systems.

Keywords: *Saccharomyces cerevisiae* | apple | biostimulant | fruit quality | yield | antioxidants

INTRODUCTION

Botanically, the apple (*Malus domestica* L.) belongs to the class *Dicotyledonae*, the family *Rosaceae*, and the genus Malus. There are approximately 30 apple species native in Asia, Europe, and North America (Özçağıran et al., 2011). Due to its wide adaptability and rich cultivar of species and types, the apple is one of the most widely cultivated fruit species in the world (Balta et al., 2020). With a significant place in horticultural cultivation, the apple has been one of the most consumed fruits in the world for over two thousand years. From an agricultural perspective, Turkey, due to its geographical and ecological conditions, is one of the rare countries where many fruit species with different climatic and soil requirements are grown together (Boyacı, 2019). The indiscriminate use of synthetic fertilizers and pesticides has negative effects on the environment and human health. Plant pathogens reduce yield and product quality, as well as posing risks to consumer health (Przybyłko et al., 2022). Resistance to pesticides reduces the effectiveness of chemical control; and the accumulation of these substances in soil and water ecosystems can disrupt ecological balance (Di Canito et al., 2021; Przybyłko et al., 2022). Pesticide use is reported to be associated with various types of cancer and poisonings in agricultural workers. For these reasons, alternative plant protection methods are needed within the framework of sustainable and organic farming approaches (Mukherjee et al., 2020).

In this process, the widespread use of microbial-based applications such as biopesticides and biofertilizers is expected; it is emphasized that microbial strains have the potential to increase plant nutrition and productivity (Mekki and Ahmed, 2005).

Citation: İleri. E.R., Özkan, Y. (2026). The Effect of Yeast (Saccharomyces cerevisiae) Application on Fruit Quality in Apples. International Journal of Agriculture, Environment and Food Sciences, 10 (1): 9–20. https://doi.org/10.66598/ijafls.1901754

In recent years, yeasts have gained importance in agriculture and are referred to as "plant growth-promoting yeasts" (PGPY) due to their effects on crop development (Nimsi et al., 2023). These microorganisms can be used as bio-stimulants or bio-fertilizers; they provide many benefits such as regulating phytohormone production, improving soil quality, facilitating nutrient uptake by plants, increasing resistance to abiotic stress conditions, and inhibiting the development of phytopathogens (Nutaratat et al., 2014; Pandi et al., 2019; Fernandez-San Millan et al., 2020; Radić et al., 2022). The application of PGPY in agricultural production is generally carried out through three main methods: seed inoculation, foliar application, and soil integration (EL-Ghamring et al., 1999; Nimsi et al., 2023). *Saccharomyces*, a genus of yeast belonging to the fungal family *Saccharomycetaceae*, is the simplest eukaryote and has been extensively observed and studied as a model organism for higher eukaryotic organisms (Replansky, Koufopanou, Greig, Bell, 2008). *Saccharomyces cerevisiae* is one of the most widely used and studied yeast species and is often known as baker's or brewer's yeast. Inactive dried yeast *Saccharomyces cerevisiae* is a natural biofertilizer effective on plants because it contains growth-promoting substances such as vitamins, amino acids, essential and secondary nutrients, and cytokinins (Bhardwaj, 2014). Dried yeast is considered a natural source of cytokinins and biostimulants that stimulate cell growth and division, chlorophyll formation, and nucleic acid and protein synthesis (Wanas, 2002; El-Desouky et al., 1998; and Wanas, 2006). Yeast extract can be effective due to its effects on biological processes, enzyme activity, photosynthetic pigments, and metabolism, which promote plant development (Wanas, 2002). It can increase antioxidant levels, improve metabolism, and enhance water retention capacity (Abbas, 2013). It also releases CO_2, which improves photosynthesis (Kurtzman and Fell, 2005). Dried yeast is used as an alternative growth nutrient source in biological and organic fertilization systems (Alsaady et. al.,2020) In this study, we aimed to investigate the effects of *Saccharomyces cerevisiae* yeast, a yeast that can play an important role in plant growth, on fruit characteristics and yields in different apple varieties.

MATERIALS AND METHODS

The study was conducted in 2024-2025 on *Mondial Gala* and *Summer Red* apple cultivars in an apple orchard belonging to the Faculty of Agriculture, Eskişehir Osmangazi University. The study material is located in Eskişehir (Central) province, which has a typical continental climate. The research was conducted on a total of 40 trees in *Mondial Gala* and *Summer Red* cultivars, with 5 replications and 1 tree per replication, using a "randomized block" statistical experimental design with 4 different doses (control (0), 5 ppm, 10 ppm and 15 ppm). *Saccharomyces cerevisiae* was applied to both varieties during their first flowering period. The application was carried out by pouring it onto the soil within a 30 cm radius of each tree's root zone. Only pure water was applied to the control plants.

***Saccharomyces cerevisiae* properties**

Saccharomyces cerevisiae, a type of budding yeast, is a single-celled yeast belonging to the phylum *Ascomycetes*. The name "*Saccharomyces*" comes from the Greek word for "sugar fungus," and "cerevisiae" from the Latin word for "beer." Other names include brewer's yeast, ale yeast, top-fermentation yeast, baker's yeast, and budding yeast (Sezgin,2010). The main organelles of S. cerevisiae are the nucleus, endoplasmic reticulum, vacuoles, mitochondria, Golgi apparatus, secretory vesicles, microbodies, ribosomes, and sometimes plasmids. The cellular contents are enclosed by the plasma membrane, periplasm, and cell Wall (Alberts, et al., 2002).

Pomological characteristics of the fruit: For scientific analyses of the fruit, 15 randomly selected fruit samples were used from each replicate, and the analyses were performed on these fruits.

Fruit weight (g): The fruits in each replicate were measured using a digital scale with a precision of 0.01 g, and their average values were calculated.

Fruit length and width (mm): The distance between the stem cavity and the blossom end of the fruit was measured using a digital caliper with a sensitivity of 0.01 mm, and the average values were calculated. The widest part of the fruit in the equatorial region was also measured using a digital caliper with a sensitivity of 0.01 mm, and the average values were calculated (Kırca,2021).

Yield per tree (g/tree): Yield per tree was determined by weighing all the product obtained from each tree.

Fruit peel color measurement (L, a, b): Fruit peel color measurements were determined using a colorimeter (Konica Minolta CR400, Japan). The L* value represented brightness-matteness; the a* value represented redness-greenness; and the b* value represented yellowness-blueness. (Krokida ve ark.,2000)

Fruit flesh firmness (kg/cm^2): The firmness of the fruit flesh was measured on both cheek regions of each replicate using a hand penetrometer (Eren et al,2005).

Soluble Solid Content (SSC) (%): Determined in fruit juices using a digital refractometer (Hanna HI96801, USA) (Eren et al,2005).

Fruit juice pH: pH was measured in fruit juices using a pH meter (Hanna Instruments, USA).

Titratable Acidity (%): The acid content of the filtered fruit juices was determined by titrating them with 0.1 N sodium hydroxide until the pH reached 8.1 on the pH meter (Küçüker, 2010).

Number of seeds (pieces):The seeds of 15 fruits from each replicate were counted manually, and the average of these measurements was taken.

Chemical analysis

Total Antioxidant Capacity (DPPH): The method determined by Brand-Williams et al. (1995) was modified and applied. For DPPH analysis, a 0.26 mM DPPH (1,1-diphenyl-2-picryl-hydrazil) solution was prepared. 2700 µL of ethyl alcohol and 1 ml of DPPH solution were added to 300 µL of fruit extract, vortexed, and then incubated in a dark environment for 30 minutes. After incubation of the samples, absorbance values were determined at 517 nm using a spectrophotometer.

Total Phenol Content: The total phenolic content was determined using Folin-Ciocalteu's chemical. According to Beyhan et al., 2010, the results were obtained by initially taking 500 µL of fresh fruit extract and adding 4.2 mL of pure water. Then, 100 µL of Folin-Ciocalteu's reagent and 2% sodium carbonate (Na_2CO_3) were added. After incubation for 1 hour, the prepared solution was measured at a wavelength of 760 nm using a spectrophotometer. The obtained absorbance values were calculated in terms of gallic acid and expressed as mgGAE 100 g-1 fw (fresh weight) (Beyhan et al., 2010).

Total Flavonoids: The total flavonoid content was determined as previously described by Kim et al. 2003. 0.3 mL of 5% $NaNO_2$ was added to 1 mL of extract and left at room temperature for 5 minutes. Then, 0.3 mL of 10% $AlCl_3$ was added, and after 1 minute, 2 mL of 1 M NaOH was added to the mixture. Immediately afterwards, 2.4 mL of pure water was added, and absorbance measurement was performed at 510 nm using a spectrophotometer. The total flavonoid content of the samples was determined using the (+)-catechin standard curve (linear range: 10–400 ppm, R2= 0.999), and the results were expressed as mg catechin equivalent (CE)/100 g CA.

Determination of vitamin C: Spectrophotometric methods were used to determine vitamin C. 2.6 The reduction of the phenol dye in dichlorophenol by ascorbic acid was utilized. For this purpose, a standard curve was obtained using solutions prepared with oxalic acid, ascorbic acid, and 2.6-D dye. Ten milliliters of fruit juice was squeezed, diluted 10 times with oxalic acid, and 1 ml was drawn into two tubes, one mixed with 9 ml of pure water and the other with 9 ml of 2.6-D dye. The obtained solutions were read at a wavelength of 518 nm in a spectrophotometer, and the corresponding ascorbic acid amount was calculated by placing the Abs value on the standard curve. Since the fruit juice was diluted 10 times, the result was multiplied by 10 and reported as x mg/100 ml (Hışıl, 1997).

Statistical Analysis

The raw data of the experiments were summed in Microsoft Excel and then transferred to SPSS 22.0 for further statistical comparison. Analysis of variance (ANOVA) was used to identify significant differences among the cultivars, and mean separation was performed using Tukey's test ($P < 0.05$). The PCA-BiPlot and Pearson correlation analysis were then carried out using the R 3.6.2 statistical program.

RESULTS AND DISCUSSION

In fruit weight measurements for the Summer Red cultivar, the control, 5 ppm, and 15 ppm applications were all in the same statistical group (b), and these doses did not have a significant effect on fruit weight ($p \leq 0.05$). In contrast, the fruit weight value increased numerically in the 10 ppm application and was placed in the letter group "ab". However, this increase did not reveal a strong statistical difference. This suggests that the Summer Red cultivar showed a limited physiological response to the application and that the application did not significantly stimulate fruit development. In contrast, the effect of the applications on fruit weight was found to be more pronounced and consistent in the Mondial Gala cultivar. In particular, the 5 ppm and 10 ppm doses (113.63g-110.61g) gave the highest fruit width values and were placed in the letter group "a", providing statistically significant increases compared to the control application. The fact that the 15 ppm application was in the "ab" group indicates that the effectiveness of the application may tend to decrease at higher doses. These findings reveal that moderate doses support fruit development in the Mondial Gala cultivar, but increasing the dose does not always produce a linear response (Table 1). In fruit width measurements of the Summer Red cultivar, the control treatment was in the "abc" group, while the 5 ppm and 15 ppm treatments were classified in the "c" group and yielded the lowest fruit width values. The 10 ppm treatment was in the "bc" group, showing a limited increase compared to these two treatments. However, no statistically significant superiority emerged between the treatments, and it was determined that the Summer Red cultivar generally showed a limited and inconsistent response to the treatments. In contrast, the effect of the treatments on fruit width was more pronounced in the Mondial Gala cultivar. In particular, the 10 ppm dose yielded the highest fruit width value and was statistically superior to all other treatments, being in the "a" group. The 5 ppm and 15 ppm treatments were in the "ab" group and provided significant increases in fruit width values compared to the control treatment. The fact that the control treatment was in the "abc" group supports the idea that these increases are treatment-dependent. These findings indicate that the Mondial Gala cultivar is more sensitive to moderate doses and that treatments within the optimum dose range have a positive effect on the fruit width parameter (Table 1). In fruit size measurements, the fact that both control and treatment groups in both varieties were in the same statistical letter group "a" indicates that there was no significant difference in fruit length between the treatments. In the Summer Red cultivar, fruit length values ranged from 53.15 mm to 55.34 mm, while in the Mondial Gala cultivar, these values ranged from 51.44mm to 55.43mm. Although small fluctuations at the numerical level were observed depending on the treatments, these changes were not found to be statistically significant (Table 1).

Table 1. Values of fruit weight (g), fruit width (mm) and fruit length (mm).

Cultivars	Application	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)
Summer Red	Control	87,76 b	58,39 abc	54,76 a
Summer Red	5 ppm	88,63 b	56,39 c	55,34 a
Summer Red	10 ppm	94,42 ab	56,95 bc	54,54 a
Summer Red	15 ppm	89,62 b	55,09 c	53,15 a
Mondial Gala	Control	99,54 ab	59,59 abc	51,44 a
Mondial Gala	5 ppm	113,63 a	62,44 ab	55,43 a
Mondial Gala	10 ppm	110,61 a	63,31 a	54,84 a
Mondial Gala	15 ppm	106,38 ab	62,53 ab	54,51 a

In their study by Karatas et al. (2021), the fruit weight of the standard summer apple cultivar Summer Red is 104 g. Similarities are also observed in the Mondial Gala cultivar. According to the study by Gündoğdu et al. (2021). The Mondial Gala cultivar was determined to have a width of 70.49, a length of 64.39, and a weight of 159.59 g. Yarılgaç (2023) and colleagues determined the fruit weight as 184.14g in 2017 and 186.66g in 2018, the fruit width as 66.13mm in 2017 and 74.31mm in 2018, and the fruit length as 55.56mm in 2017 and 73.05mm in 2018. Bayazıt (2018) and colleagues determined the fruit weight as 232.2g, the fruit width as 77.66mm, and the fruit length as 59.83mm.

In the Summer Red cultivar, the 10 ppm application yielded the highest seed count, placing it in group "a" and statistically superior to other applications. Conversely, the 15 ppm application showed the lowest seed count, placing it in group "b". The fact that the control and 5 ppm applications were in group "ab" indicates that these applications did not statistically differ in either the highest or lowest values. These findings show that the seed count parameter in the Summer Red cultivar exhibits a significant response to doses, with moderate doses particularly having an enhancing effect on this parameter. However, the decrease observed at the high dose (15 ppm) suggests that increasing the dose may have a negative impact after a certain threshold. In contrast, in the Mondial Gala cultivar, all applications were in group "ab", indicating that there was no statistically significant difference between applications in terms of the seed count parameter. Although the lowest numerical value was obtained with the 5 ppm application, this decrease was not statistically significant. This suggests that the seed number parameter in the Mondial Gala cultivar exhibits a more stable character against treatments, and that genotypic factors play a more dominant role in this trait (Table 2).

In fruit firmness measurements, the fact that both control and treatment groups in both varieties were statistically classified in the same letter group "a" indicates that there was no significant difference in firmness between the treatments. In the Summer

Red cultivar, fruit firmness values ranged from 7.68 to 7.23, while in the Mondial Gala cultivar, these values ranged from 7.71 to 7.16 (Table 2).

Table 2. The number of seeds and fruit hardness values are given.

Cultivar	Application	Number of seed	Fruit flesh firmness
Summer Red	Control	4,72 ab	7,24 a
Summer Red	5 ppm	4,48 ab	7,42 a
Summer Red	10 ppm	5,68 a	7,68 a
Summer Red	15 ppm	3,28 b	7,23 a
Mondial Gala	Control	4,88 ab	7,71 a
Mondial Gala	5 ppm	3,64 ab	7,16 a
Mondial Gala	10 ppm	4,67 ab	7,38 a
Mondial Gala	15 ppm	4,19 ab	7,61 a

In their studies conducted on the Mondial Gala cultivar determined that, according to the study by Bayazıt et al. (2018), the number of kernels is 6 and the hardness is 1.29 lb. Karatas (2021) et al. determined the fruit firmness of the Summer Red cultivar as 5.66 kg/cm.

According to pH measurements in the study, no difference was observed between the application groups in the Summer Red cultivar. All application results falling into the "c" group indicate no significant difference in pH for the Summer Red cultivar. In the Mondial Gala cultivar, the best pH level was observed at a dose of 15 ppm. The lowest pH was observed in the control group (Table 3).

In the Summer Red cultivar, the control and 5 ppm applications were classified in the "abc" letter group, and no statistically significant difference was found in their soluble dry matter (SSC) values. In contrast, the 10 ppm application was in the "bc" group, and the 15 ppm application was in the "c" group, showing lower SSC values compared to the control application. The decrease observed particularly at the 15 ppm dose suggests that high-dose applications may negatively affect the accumulation of soluble dry matter in the Summer Red cultivar. These findings indicate a regular decrease in SSC values with increasing doses, suggesting that the Summer Red cultivar may be sensitive to high doses. In the Mondial Gala cultivar, the control and 15 ppm applications were classified in the "a" letter group, producing the highest SSC values. The 5 ppm and 10 ppm applications were classified in the "ab" group and showed no statistically significant difference compared to the control application. These results show that the applications caused limited changes in SSC in the Mondial Gala cultivar, but the 15 ppm dose in particular had a protective or enhancing effect on SSC values (Table 3).

In the measurements of titratable acidity, all application groups for the Summer Red cultivar are in the letter group "a". Similarly, all application for the Mondial Gala cultivar are in the letter group "b". While small numerical fluctuations were observed depending on the application for both Summer Red and Mondial Gala, these variations were not statistically significant. (Table 3).

Table 3. pH, SSC (soluble solids content), and TEA (titratable acidity) values are provided.

Cultivar	Application	pH	SSC	TA (%)
Summer Red	Control	4,77 c	11,26 abc	1,12 a
Summer Red	5 ppm	4,75 c	11,42 abc	1,11 a
Summer Red	10 ppm	4,79 c	10,56 bc	0,95 a
Summer Red	15 ppm	4,74 c	9,72 c	0,85 a
Mondial Gala	Control	5,09 b	12,90 a	0,36 b
Mondial Gala	5 ppm	5,18 ab	12,16 ab	0,34 b
Mondial Gala	10 ppm	5,16 ab	12,02 ab	0,32b
Mondial Gala	15 ppm	5,25 a	12,86 a	0,33b

In the study conducted by Bayazıt et al. (2018) on the Mondial Gala cultivar, the average pH was calculated as 4.08 and the titratable acidity as 0.93%. In the study conducted by Yargıç et al. (2023) in 2017 and 2018, the average pH was determined as

4.12, SSC as 12.55 and TA as 0.35%. In the study conducted by Gündoğdu et al. (2021), the pH was determined as 3.79 and SSC as 12.86. Karakaş (2022) also determined the SSC value as 12.10 in his study.

When the L* values were examined, the Mondial Gala cultivar showed higher lightness values compared to the Summer Red cultivar in all applications. This indicates that Mondial Gala has a lighter and brighter peel color. No statistically significant difference was found in L* values between application doses in either cultivar. This result suggests that the applications may affect color tones and pigment distribution rather than fruit peel lightness. In terms of the a* value (redness) parameter, the Mondial Gala cultivar reached significantly higher values than Summer Red. In Mondial Gala, the control, 10 ppm, and 15 ppm applications were in the "a" letter group, maintaining a high redness level. In the Summer Red cultivar, the decrease in the a* value at the 15 ppm application and its placement in the "b" letter group suggests that high-dose application may suppress red color intensity. This may be related to the dose-dependent negative impact on anthocyanin synthesis. b* Value (Yellowing): When the b* values were examined, the Mondial Gala cultivar was statistically placed in the "a" group in all applications and showed higher yellowing values. The Summer Red cultivar was in the "b" group in all applications. This result reveals that Mondial Gala has a lighter background color and a bark color where yellow tones are more dominant. It was determined that the application doses did not have a significant effect on the b* value in either cultivar. (Table 4).

Table 4. Color measurements L, a, b values are given.

Cultivar	Application	L*	a*	b*
Summer Red	Control	52,78 b	21,30 ab	22,87 b
Summer Red	5 ppm	51,90 b	23,18 ab	21,07 b
Summer Red	10 ppm	52,53 b	23,34 ab	22,25 b
Summer Red	15 ppm	51,32 b	17,74 b	23,28 b
Mondial Gala	Control	60,02 a	29,44 a	27,68 a
Mondial Gala	5 ppm	63,70 a	23,85 ab	28,34 a
Mondial Gala	10 ppm	58,98 a	29,18 a	28,59 a
Mondial Gala	15 ppm	60,94 a	27,68 a	27,78 a

In their study conducted by Yarılgaç (2023) and colleagues on the Mondial Gala cultivar, the color parameters were determined as an average of 56.74 L*, 23.60 a*, and 26.95 b* for the years 2017 and 2018. In the study conducted by Bayazıt (2018) and colleagues, the L* value was determined as 54.70, the a* value as 12.07, and the b* value as 26.35.

In the Summer Red cultivar, the highest yield value was determined with the 5 ppm application (4266.28g), placing it in the "a" group and showing statistical superiority compared to other applications. The control, 10 ppm, and 15 ppm applications were in the "ab" group, and no statistically significant difference was found between these applications. This indicates that the low dose application had a yield-enhancing effect in the Summer Red cultivar, but higher doses did not provide an additional advantage. In the Mondial Gala cultivar, the 10 ppm application showed the highest yield value (3182.16g) and was placed in the "ab" group along with the control group. In contrast, the 5 ppm (1475.70g) and 15 ppm (1030.24g) applications were in the "b" group, showing lower yield values. These results suggest that low and high dose applications may negatively affect the yield of the Mondial Gala cultivar, while the medium dose has a relatively more favorable effect.

In the Summer Red cultivar, flavonoid values ranged from 87.80 mg/QE/100g to 100.84 mg/QE/100g, with the control and all application doses belonging to the same statistical group (b). This result indicates that the applications did not cause a significant increase or decrease in flavonoid synthesis in the Summer Red cultivar. However, although the highest numerical flavonoid value was determined in the 10 ppm application, this increase was not statistically significant. This suggests that flavonoid biosynthesis may be genetically limited for this cultivar. In the Mondial Gala cultivar, the control application showed the highest flavonoid content with a value of 145.26 mg/QE/100g and was statistically placed in group "a". In contrast, a significant decrease in flavonoid content occurred in the 5 ppm and 10 ppm applications, and these applications were classified in group "b". The 15 ppm application showed an intermediate level with a value of 117.90 mg/QE/100g and was placed in group "ab". These findings reveal that flavonoid accumulation in the Mondial Gala cultivar is more sensitive to treatments, and that low and medium doses, in particular, can have a suppressive effect on flavonoid synthesis.

Table 5. Yield per tree.

Cultivar	Application	Yield (g/tree)
Summer Red	Control	1847,48 ab
Summer Red	5 ppm	4266,28 a
Summer Red	10 ppm	2568,54 ab
Summer Red	15 ppm	2133,92 ab
Mondial Gala	Control	1690,50 ab
Mondial Gala	5 ppm	1475,70 b
Mondial Gala	10 ppm	3182,16 ab
Mondial Gala	15 ppm	1030,24 b

In the Summer Red cultivar, the highest AsA value was determined in the 5 ppm application (3.76 mg/100ml), placing it in the letter group "a" and statistically superior to other applications. The lowest AsA value (2.09 mg/100ml) was detected in the control application, placing it in the letter group "b". The 10 ppm (2.72 mg/100ml) and 15 ppm (2.30 mg/100ml) applications were in the letter group "ab", showing partial similarity to both the control and 5 ppm applications. This suggests that the low-dose application promotes ascorbic acid accumulation in the Summer Red cultivar, but increasing the dose limits this effect. In the Mondial Gala cultivar, AsA contents were generally lower compared to the Summer Red cultivar. The highest AsA value was obtained in the control application (2.46), placing it in the letter group "ab" along with the 5 ppm application (2.24 mg/100ml). In contrast, at 10 ppm (1.88 mg/100ml) and especially 15 ppm (1.30 mg/100ml) applications, the AsA content was significantly reduced and was classified in the letter group “b”. These findings indicate that increasing doses have a suppressive effect on ascorbic acid content in the Mondial Gala cultivar.

In the Summer Red cultivar, the lowest TPC value was determined in the control application (116.40 mg/GAE L), placing it in the letter group "c". In contrast, the 5 ppm application provided the highest TPC content with a value of 241.20, placing it in the letter group "a" and showing statistical superiority over the other applications. The 10 ppm application (149.60 mg/GAE L) was in the letter group "bc" and showed partial similarity to the control. In the 15 ppm application, the TPC value was determined as 183.20 mg/GAE L, placing it in the letter group "abc" and showing a moderate increase. These findings indicate that in the Summer Red cultivar, low-dose application significantly promotes phenolic compound accumulation, but increasing the dose limits this positive effect. In the Mondial Gala cultivar, TPC values were generally higher compared to the Summer Red cultivar. The control (262.60 mg/GAE L), 5 ppm (239.20 mg/GAE L), and 10 ppm (243.00 mg/GAE L) applications were in the same statistical group ("a"), and no significant difference was found between them. In contrast, the 15 ppm application (215.60 mg/GAE L) showed a relative decrease compared to other applications, placing it in the “ab” letter group. This suggests that the phenolic compound content in the Mondial Gala cultivar exhibits a more stable structure against the applications, but that phenolic metabolism can be partially suppressed at high doses.

In the Summer Red cultivar, DPPH activity generally remained at low levels, and no significant increase was observed between treatments. The lowest DPPH value was determined in the 5 ppm treatment (6.60%), placing it in group "c" and showing statistically lower values compared to other treatments. The control (17.78%), 10 ppm (21.80%), and 15 ppm (16.50%) treatments were in group "bc," and no significant difference was found between them. These results indicate that the treatments had a limited effect on the DPPH radical scavenging capacity of the Summer Red cultivar. In contrast, the Mondial Gala cultivar showed a more pronounced response to the treatments in terms of DPPH activity. The highest DPPH values were obtained in the 5 ppm (56.74%) and 15 ppm (52.30%) treatments, both placing them in group "a" and showing statistical superiority. The 10 ppm treatment (29.78%) was in group "b" and showed moderate antioxidant activity. The control treatment (23,46%) remained at a low level, belonging to the letter group “bc”. These findings indicate that in the Mondial Gala cultivar, certain doses significantly increase the DPPH radical scavenging capacity by stimulating antioxidant defense mechanisms.

Table 6. Chemical measurements, Flavonoids, Total phenolic compounds, Vitamin C determination, Total antioxidant capacity (DPPH)

Cultivar	Application	Flavonoid (mg/QE/100g)	TPC (mg/GAE L)	AsA (mg/100ml)	DPPH (%)
Summer Red	Control	87,80 b	116,40 c	2,09 b	17,78 bc
Summer Red	5 ppm	98,78 b	241,20 a	3,76 a	6,60 c
Summer Red	10 ppm	100,84 b	149,60 bc	2,72 ab	21,80 bc
Summer Red	15 ppm	92,58 b	183,20 abc	2,30 ab	16,50 bc
Mondial Gala	Control	145,26 a	262,60 a	2,46 ab	23,46 bc
Mondial Gala	5 ppm	97,42 b	239,20 a	2,24 ab	56,74 a
Mondial Gala	10 ppm	93,50 b	243,00 a	1,88 b	29,78 b
Mondial Gala	15 ppm	117,90 ab	215,60 ab	1,30 b	52,30 a

Principal Component Analysis (PCA) – Biplot Interpretation

According to the principal component analysis (PCA) results, the first two principal components explained 48.3% of the total variance. The first principal component (Dim1) represented 31.4% of the total variance, and the second principal component (Dim2) represented 16.9%. These percentages indicate that a significant portion of the measured pomological, chemical, and biochemical characteristics can be reliably summarized in a two-dimensional plane.

The Dim1 axis is predominantly defined by fruit quality and chemical properties. Specifically, fruit weight (FW), fruit length (FL), pH, soluble dry matter content (SSC), total phenolic content (TPC), flavonoid content (Flav), DPPH radical scavenging activity, and color parameters (L*, a*, b*) exhibited high positive loadings along the Dim1 axis. This indicates that these variables are positively correlated with each other and act together as key factors determining quality. In contrast, the vectors of total acidity (TA) and ascorbic acid (AsA) appear to be located in the negative direction of the Dim1 axis. This distribution suggests that acidity and vitamin C content are inversely related to sugar accumulation and phenolic compounds, and are controlled by different biochemical mechanisms during the maturation process.

The Dim2 axis is more closely related to yield, seed number (SN), and fruit firmness (firm). The fact that yield and seed number are located on the positive side of Dim2 suggests that this axis is related to production potential. However, the more limited contribution of quality parameters along Dim2 reveals that yield and quality characteristics do not always follow a parallel trend. When the distribution of cultivar × application combinations is examined, it is observed that applications belonging to the MS cultivar are generally concentrated in the positive region of Dim1. This indicates that the MS cultivar has higher values, especially in terms of fruit size, sugar content, color parameters, and antioxidant capacity. The fact that MS 5 and MS 10 ppm applications are positioned in the same direction as the quality parameters suggests that these doses may be more effective in terms of quality. It was determined that the applications belonging to the SR cultivar were predominantly located in the negative direction of Dim1 and closer to the TA and AsA vectors. This result reveals that the applications in the SR cultivar produced a response more related to acidity and ascorbic acid content, and that the quality profile exhibited a different biochemical character than the MS cultivar. In particular, the dissociation of the SR 15 ppm application along Dim2 indicates that this application may have a significant effect on yield and cluster number. Overall, PCA-Biplot analysis revealed that cultivar and treatment combinations clearly differentiated in terms of fruit quality components and yield parameters. The findings clearly demonstrate that the effect of treatments varies depending on the cultivar and that quality andyield characteristics should be evaluated in a multidimensional structure. Therefore, PCA results can be considered an important decision support tool for determining appropriatecultivar-dose combinations in cultivation.

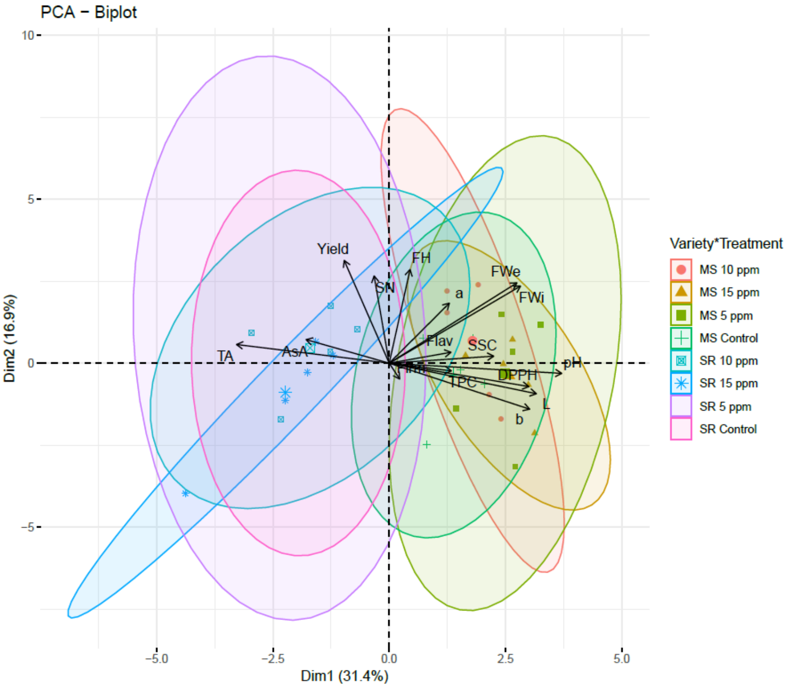
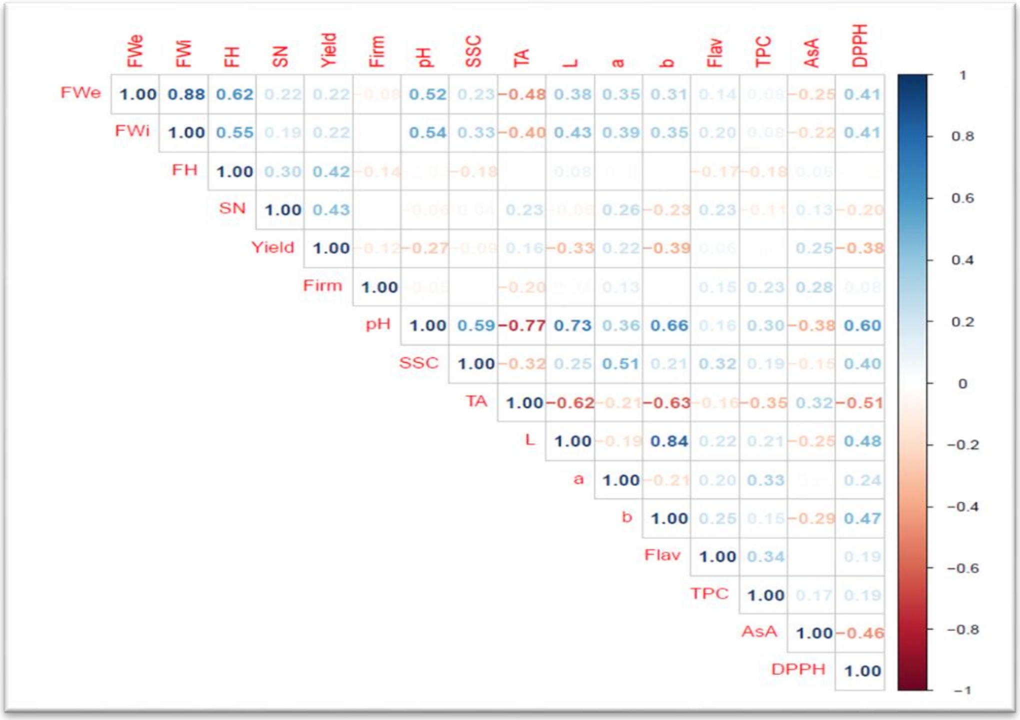


Figure 1. Biplot analysis

Evaluation of Correlation Analysis

Correlation analysis results show significant and biologically consistent relationships between pomological, chemical, and biochemical characteristics. Strong and positive correlations were found particularly between physical size parameters of the fruit (fruit width, and fruit length) (r = 0.55–0.88). This indicates that fruit weight and size increase together and that these parameters are complementary. Similarly, a moderately positive relationship (r ≈ 0.52–0.54) was determined between fruit weight and pH, suggesting that larger fruits may have relatively higher pH values. The positive correlation between soluble dry matter content (SSC) and pH (r = 0.59), and the strong negative correlation between total acidity (TA) and pH (r = −0.77), demonstrate that fruit taste balance is mutually controlled by the basic chemical parameters. The negative relationship between SSC and TA is consistent with the increase in sugar accumulation and decrease in acidity as the ripening process progresses. These results support the necessity of evaluating SSC, pH, and TA together in determining fruit ripeness indices. When color parameters were examined, a high level of positive correlation (r = 0.84) was found between L* and a* values. This indicates that fruit brightness and redness tone change together, and that pigment formation plays a decisive role in color perception. The b* value, on the other hand, showed weak-to-moderate correlations with both SSC and antioxidant properties, revealing that color formation is related not only to sugar content but also to biochemical components. The positive correlation between phenolic compounds (TPC) and flavonoid content (Flav) (r ≈ 0.34) indicates a structural and biosynthetic relationship between these two components of phenolic metabolism. The significant negative correlation (r = −0.46) determined between ascorbic acid (AsA) and DPPH suggests that antioxidant capacity is not dependent on a single component, but that different antioxidant systems act together. In contrast, the observed positive correlation between DPPH and pH (r = 0.60) indicates that radical scavenging capacity increases under higher pH conditions. The relationships between yield and quality parameters are generally weak to moderate, with negative trends observed particularly between yield and total acidity and color parameters. This reveals that high yield does not always parallel superior fruit quality and that the yield-quality balance should be considered in cultivation practices. Overall, the correlation analysis results show that the physical, chemical, and biochemical properties of the fruit are largely interrelated, but the direction and strength of these relationships vary depending on the variables. The findings clearly demonstrate that quality formation exhibits a multifactorial structure and that evaluation based on a single parameter is insufficient.



	FWe	FWi	FH	SN	Yield	Firm	pH	SSC	TA	L	a	b	Flav	TPC	AsA	DPPH
FWe	1.00	0.88	0.62	0.22	0.22	−0.08	0.52	0.23	−0.48	0.38	0.35	0.31	0.14	0.08	−0.25	0.41
FWi		1.00	0.55	0.19	0.22		0.54	0.33	−0.40	0.43	0.39	0.35	0.20	0.08	−0.22	0.41
FH			1.00	0.30	0.42	−0.14	[illegible]	−0.18		0.08	[illegible]		−0.17	−0.18	0.06	[illegible]
SN				1.00	0.43		−0.06	0.04	0.23	−0.06	0.26	−0.23	0.23	−0.11	0.13	−0.20
Yield					1.00	−0.12	−0.27	−0.09	0.16	−0.33	0.22	−0.39	0.06	[illegible]	0.25	−0.38
Firm						1.00	−0.05		−0.20	0.04	0.13		0.15	0.23	0.28	0.08
pH							1.00	0.59	−0.77	0.73	0.36	0.66	0.16	0.30	−0.38	0.60
SSC								1.00	−0.32	0.25	0.51	0.21	0.32	0.19	−0.15	0.40
TA									1.00	−0.62	−0.21	−0.63	−0.16	−0.35	0.32	−0.51
L										1.00	−0.19	0.84	0.22	0.21	−0.25	0.48
a											1.00	−0.21	0.20	0.33	[illegible]	0.24
b												1.00	0.25	0.15	−0.29	0.47
Flav													1.00	0.34		0.19
TPC														1.00	0.17	0.19
AsA															1.00	−0.46
DPPH																1.00

Figure 2. Correlation analysis.

CONCLUSIONS

This study evaluated the effects of different doses of regulators on pomological characteristics, physicochemical quality parameters, and antioxidant compounds in Summer Red and Mondial Gala apple varieties. The results showed that the effects of the applications were largely cultivar and dose-dependent.

In terms of pomological characteristics, the Summer Red cultivar showed a limited and inconsistent response to the treatments. No significant differences were found between treatments in fruit weight, fruit width, and fruit length parameters; only a significant increase was observed in seed number at the 10 ppm dose. In contrast, in the Mondial Gala cultivar, the 5 ppm and 10 ppm doses increased fruit weight and fruit width values, while at higher doses (15 ppm), the effectiveness tended to decrease. Fruit firmness was not affected by the treatments in either cultivar.

When the physicochemical quality parameters were examined, no significant improvement was observed in pH, titratable acidity, and water-soluble dry matter values in the Summer Red cultivar depending on the application; a downward trend in SSC values was determined at high doses. In the Mondial Gala cultivar, however, it was found that SSC and pH values were generally more stable and could be maintained especially at a dose of 15 ppm.

In terms of color parameters, the Mondial Gala cultivar showed higher lightness (L*) and redness (a*) values compared to Summer Red. The decrease in the a* value at 15 ppm application in the Summer Red cultivar suggests that higher doses may suppress color formation.

Biochemical analyses showed that low-dose (5 ppm) application increased ascorbic acid and total phenolic content in the Summer Red cultivar, but had a limited effect on flavonoid and DPPH activity. In the Mondial Gala cultivar, it was determined that increasing doses suppressed ascorbic acid and flavonoid content, while significantly increasing DPPH radical scavenging capacity at certain doses.

Overall, the Mondial Gala cultivar showed a more responsive and consistent reaction to the treatments, while in the Summer Red cultivar, low and medium doses had limited positive effects on some quality and antioxidant parameters. These results indicate that the optimum dose for apple varieties should be determined genotype-specifically, and that high-dose applications do not always yield positive results.

Acknowledgement

This study is a part of the master thesis of the first author. The study was supported by Scientific Research Projects Coordination Unit of Eskisehir Osmangazi University, Project code: FYL-2025-3410. The authors are grateful to Dr. İbrahim Kahramanoğlu, Prof. at European University of Lefke for his valuable helps in the statistical analysis

Author Contributions

All authors contributed to the emergence of the manuscript and approved the final version

Conflict of Interest

The authors declare that they have no conflict of interest.

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