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A comparative Study on Improving Balady Mandarin Growth, Yield and Fruit Quality with Foliar Antioxidant in Semi-Dry Conditions

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Abstract

A field experiment was conducted over two growing seasons (2021 and 2022) to assess the impact of various treatments on the morphological traits, yield, and chemical composition of Balady mandarin trees. The experiment encompassed 51 fifteen-year-old trees planted with common horticultural practices. Sixteen treatments, including citric acid, vitamin A+E, salicylic acid, and vitamin B complex, were applied at four concentrations (200, 400, 800, and 1600 ppm), alongside a control treatment. The results revealed significant effects on morphological traits, with 1600 ppm of citric acid leading to an increase in leaf area (109.12 and 98.19 %) more than the control treatment in first and second seasons, respectively. Fruit yield was influenced by these treatments, with 1600 ppm of citric acid resulting in the highest number of fruits per tree (492 and 580) and the heaviest average fruit weight (139.03 and 145 g.) in first and second seasons, respectively. Additionally, trees treated with 1600 ppm of citric acid had the highest yield per tree (75.43 and 73.63 kg) and the lowest percentage of pre-harvest fruit dropping (21.90 and 21 %), in first and second seasons, respectively, indicating improved fruit retention. Chemical composition analysis of fruit juice demonstrated that citric acid treatments produced fruit with higher total soluble solids (13.73 and 13.7), reducing sugars (9.2 and 9.43 %), and total sugars (10.13 and 10.23 %), as well as elevated levels of vitamin C (45.46 and 45.03) in first and second seasons, respectively. Control trees exhibited lower values for these attributes. Overall, the study highlights the potential of these treatments, particularly citric acid at 1600 ppm, to enhance the growth, yield, and fruit quality of Balady mandarin trees in Egyptian orchards. These findings offer valuable insights for citrus orchard management practices and fruit production optimization.

Keywords: Citrus; plant growth; Pre-harvest fruit dropping; Total soluble solids; Soluble solids; Sugar-acid composition.

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1. Introduction

Citrus is a highly significant fruit crop worldwide, with particular prominence in the United States and warm temperate regions. It has also thrived successfully in Egypt [1]. In Egypt, citrus cultivation takes center stage in the fruit crop landscape, holding the top position among all other fruits [1]. Approximately 29% of the total fruit-growing area, equivalent to 204,095 hectares out of 700,854 hectares, is dedicated to citrus cultivation [2]. These citrus orchards, totaling 175,734 hectares, primarily utilize Nile River freshwater for irrigation in the Delta region [2]. Mandarin (*Citrus reticulata* L. Blanco) ranks among the most important citrus fruits in various countries, including Egypt. In Egypt, the predominant mandarin production revolves around local cultivars like Balady, which belong to the common Mediterranean mandarin group [3]. Antioxidants help protect fruit trees from oxidative stress [4]. Just as in humans, oxidative stress in plants can result from an imbalance between harmful reactive oxygen species (ROS) and the plant's antioxidant defenses [5]. Antioxidants can improve the quality of the fruit produced by the tree, helping maintain its color, texture, and flavor [6]. Additionally, antioxidants can slow down the deterioration of fruits after harvest, extending their shelf life and marketability [7]. By reducing oxidative stress and protecting against environmental stressors like heat, drought, and disease, antioxidants can promote overall tree health [8]. Healthy trees are more likely to bear higher yields of fruit [9]. Some antioxidants can also contribute to the tree's resistance to diseases and pests [10]. While they are not a replacement for traditional pest and disease control measures, antioxidants can support the tree's natural defense mechanisms. Using antioxidants in fruit tree cultivation can be part of a sustainable and environmentally friendly agricultural approach. It can reduce the need for chemical pesticides and fertilizers, leading to less environmental impact. Citric acid serves as a plant metabolite and plays a crucial role in both photosynthesis and cellular respiration [11, 12]. One of its noteworthy effects in food is the enhancement of antioxidant properties by promoting the increased production of nutraceutical compounds, including phenolic compounds and flavonoids [13]. These compounds work synergistically, influencing the growth, yield, and certain chemical constituents in numerous crops. Additionally, they exert dominance over the occurrence of most fungi affecting various crops [14].

Vitamin A and E are antioxidants that help promote plant growth and development [15]. Vitamin A and E contribute to encouraging the formation of tissues and enzymes necessary for growth and development [15]. It can reduce the effects of environmental stress such as pigmentation, root damage and dryness. Crops sprayed with vitamins A and E have a better chance of producing crops of higher quality and greater quantities [16]. Vitamin E plays an important role in enhancing plants' resistance to diseases and pests by enhancing the plant's natural defense system [17]. Spraying plants with vitamins E contributes to improving the quality of the final agricultural product [18]. Salicylic acid plays a pivotal role in mitigating stress when plants are exposed to adverse conditions, aiding in the activation of the antioxidant system. It is now recognized as a hormonal substance with significant involvement in the regulation of plant growth and development [19]. Vitamin B complex, which includes B1 (thiamine), B2 (riboflavin), and B3 (niacin), offers significant benefits to plants by supporting essential metabolic processes. Thiamine (B1) plays a pivotal role in carbohydrate metabolism and energy production within plant cells [20]. Riboflavin (B2) is essential for photosynthesis and helps in the activation of enzymes involved in the breakdown of sugars [21]. Niacin (B3) contributes to the production of coenzymes that facilitate various biochemical reactions crucial for plant growth, including the synthesis of DNA and RNA [22]. In the study conducted by Dawood, Abdel-Baky [23], it was determined that nicotinamide had a favorable impact on growth parameters, photosynthetic pigments, seed yield, and various components contributing to yield in faba bean seeds. Additionally, these B vitamins aid in the plant's defense mechanisms against environmental stressors [24] and promote overall plant health [25]. By ensuring proper metabolic functions and stress resistance, vitamin B complex helps optimize plant growth, vigor, and crop yields, making it a valuable component of plant nutrition and agriculture [26].

The main goal of this study is to assess how specific antioxidants (citric acid, vitamin A+E, salicylic acid, and vitamin B complex) affect the morphological measurements, chemical characteristics of fruits, and yield of Balady mandarin trees. The hypothesis was spraying Balady mandarin trees with these treatments would increase leaf area and vitamin C content compared to the control group. Furthermore, could be predicted that these treatments would increase in total yield due to their potential to improve fruit set and reduce pre-harvest fruit drop. This positive impact on yield and fruit quality might be mediated by increased levels of antioxidant compounds within the fruit induced by some of these treatments.

2. Materials and Methods

2.1. Experimental design and treatments

The plant experiments adhered to both local and national regulations and adhered to the guidelines of Al-Azhar University's Assiut branch in Assiut, Egypt. We obtained authorization for our research to conform to the appropriate institutional, national, and international standards and laws. A field experiment was conducted in a private orchard located in An Nikhaylah Village, Abu Tig City, Assiut Governorate, Egypt (longitude 31.3459824 and latitude 27.0155822), spanning the 2021 and 2022 growing seasons. Based on the Köppen–Geiger {Beck, 2018 #41} climate classification map, Abu Tig city in Egypt experiences extremely hot and arid summers, and chilly winters. Figure 1 illustrates the maximum and minimum temperatures as well as the relative humidity throughout the two growing seasons. The study focused on fifteen-year-old Balady mandarin trees (*Citrus reticulata* L. Blanco) grafted onto sour orange rootstock. These trees were planted at a spacing of 5 x 5 m, and a surface irrigation system was employed for their care. Common horticultural practices were implemented as standard procedure including pruning and pest and disease management. The experiment encompassed a total of 51 trees, all carefully selected based on their uniformity in terms of healthy growth, fruiting, flowering behavior, and adherence to the same cultural practices. Fertilization followed the recommendations provided by the Ministry of Agriculture, Egypt for each tree [In the spring: 20 kg of balanced organic fertilizer + 500 g of ammonium nitrate + 200 g of calcium superphosphate + 100 g of potassium sulfate; In the summer: 20 kg of balanced organic fertilizer + 200 g of calcium superphosphate + 100 g of potassium sulfate; In the fall: 500 g of calcium superphosphate + 200 g of potassium sulfate; In winter: 20 kg of balanced organic fertilizer [27].

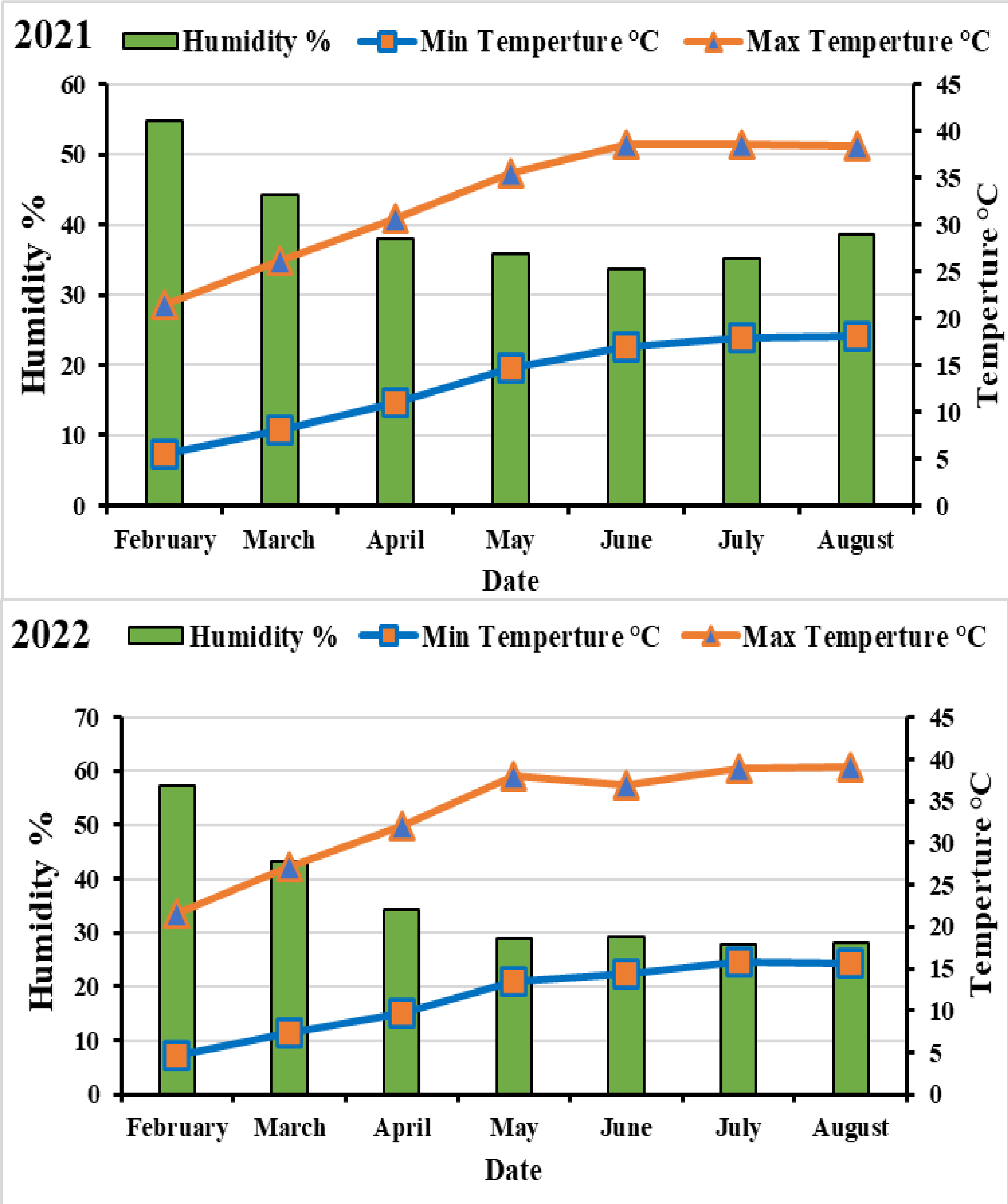


Figure 1. Weather conditions [Maximum temperature, Minimum temperature, and Relative humidity] during the growing seasons (2021 and 2022) of Balady mandarin.

Table 1. Displays various physical and chemical attributes of the experimental soil

Parameters			Values	
			2021	2022
Particle size distribution	Sand (%)		5.0	5.0
	Silt (%)		55.0	55.0
	Clay (%)		40	40
	Texture grade		Silt clay	Silt clay
Macro nutrients	Available (mg kg^{-1})	N	90.09	80.09
		P	15.2	18.2
		K	402.2	382.2
Chemical properties	pH (Susp. 1:2.5 soil-water)		7.92	8
	EC_e (1:1 dSm^{-1})		1.72	1.48
	Organic matter (g kg^{-1})		1.42	2.0
	SOC (%)		0.824	1.160
	$CaCO_3$ (g kg^{-1})		2.22	3.5

The values presented in the table represent the average of four replicates for each measurement.

The experiment entailed the use of four different treatments, namely citric acid, vitamin A+E, salicylic acid, and vitamin B complex. These treatments were applied at 4 concentrations each (200, 400, 800, and 1600 ppm), in addition to a control treatment involving water spraying. Consequently, the experiment comprised a total of seventeen treatments. All treatment sprays were carried out three times: at the beginning of growth (last week of February), after fruit set (first week of April), and 2 months after the second spraying (first week of June). A 1% solution of Triton B, a wetting agent, was incorporated into all spraying solutions. Each tree received foliar application until runoff, equivalent to 10 L per tree. The control treatment involved the application of tap water. The experimental design employed was a randomized complete block design, consisting of three replicates, with each replicate represented by one tree.

2.2. Soil and plant analysis

The soil texture was categorized as silty clay (as shown in Table 1). In each season, samples were randomly collected from two depths, 0-30 cm and 30-60 cm below the soil surface, for both physical and chemical analyses [28, 29]. Upon arrival at the laboratory, the soil samples underwent a series of preparatory steps. Initially, the samples were dried in an oven at 40°C, then crushed to pass through a 2 mm sieve, and finally ground to achieve a particle size of less than 60 μm. These procedures followed the methodology outlined by Madejón, Marañón [30]. Soil texture was determined using the pipette method, as outlined by Page et al. (1982). To assess the soil's characteristics, the pH was measured by creating a 1:2.5 soil-to-water suspension and using a Beckman pH meter. Additionally, electrical conductivity (EC) was measured at a 1:2.5 soil-to-water ratio using a salt bridge, following method of Jackson [31]. The total calcium carbonate content ($CaCO_3$) was determined using a Collins calcimeter, employing the procedure outlined by Loeppert and Suarez [32]. For the determination of soil organic carbon content (SOC%), Walkley and Black's wet oxidation method was utilized. This method involves the use of $K_2Cr_2O_7$ (1N) and concentrated H_2SO_4, followed by titration using standardized 0.5 M $(NH_4)_2SO_4.FeSO_4.6H_2O$, as described by Walkley and Black [33]. For the assessment of available nitrogen (N), the soil samples were subjected to extraction with a 1% K_2SO_4 solution at a ratio of 1:10. Five milliliters of the resulting extract were distilled, with the addition of 0.1 g of a mixture consisting of magnesium oxide (MgO) and Devarda's alloy. This distillation process was carried out using a micro Kjeldahl's distilling unit, following Jackson [31] protocol. The distillate was collected in an Erlenmeyer flask containing 15 mL of boric acid (H_3BO_3) combined with an indicator solution, with approximately 50 mL of distillate collected in each flask. The determination of available nitrogen content (comprising NH_4^+ and NO_3^-) in the distillate was accomplished through titration with standardized 0.01 N sulfuric acid, as described by Jackson [31]. For the evaluation of available phosphorus (P), the soil samples were extracted with 0.5 M ($NaHCO_3$) at pH 8.5, following a 1:10 ratio. The extracted P was quantified using a spectrophotometer (JENWAY 6305 UV/Visible spectrophotometer, U.K.) with the stannous chloride phosphomolybdic-sulfuric acid system, in accordance with Jackson [31] method. Available potassium (K) was extracted using 1 M ammonium acetate at pH 7, also at a 1:10 ratio. The extracted K was measured through flame photometry using a BWB model BWB-XP, 5-channel, JENWAY, model: PFP7, U.K., as per Jackson [31] guidelines.

2.3. Data collection

2.3.1. Leaf area

The growth cycle of leaf area (in square centimeters, cm²) was assessed based on the methodology described by Ahmed and Morsy [34]. Three replicates each replicate has twenty mature leaves were selected for measurement during the first week of September. The leaf area was determined using the following equation: LA = 0.49 × (L × W) + 19.09. Here, LA represents leaf area in cm², L stands for the length of the leaf, and W represents the width of the leaf.

2.3.2. Fruit Yield

During the maturity stage, which occurred around the middle of December, several assessments were conducted. The average number of fruits per tree was determined by counting fruits of three trees each tree as a replicate. Additionally, the average fruit weight was calculated by weighing a sample of five fruits from each replicate (selected tree), and this information was used to calculate the average fruit weight in grams. To obtain the average yield per tree in kilograms, this average fruit weight was then multiplied by the average number of fruits per tree. Furthermore, the percentage of pre-harvest fruit dropping was computed using the formula: ((Number of total fruit set - Number of fruits at the last count) / Number of total fruits set) * 100.

2.3.3. Percentage of Pre-harvest Fruit Dropping

The dropped fruits and the total number of fruits that completed their growth on the tree were counted, and the following formula was used to calculate the percentage of fruits that dropped before harvest:

$$Percentage\ of\ Pre-harvest\ Fruit\ Dropping\ (\%) = \frac{Number\ of\ Dropped\ Fruits}{(Number\ of\ Dropped\ Fruits\ +\ Total\ number\ of\ fruits\ that\ completed\ their\ growth\ on\ the\ tree)} \times 100$$

2.3.4. Chemical composition analysis of fruit

At harvesting time randomly sample of five fruits /tree (replicate) was collected. Firstly, the mandarin fruits were juiced, and the juice was filtered to remove any solid particles or pulp. The filtered juice was then diluted with distilled water to an appropriate concentration for the titration. After that determined the following:

2.3.4.1. Total soluble solids (TSS) in the mandarin fruit juice was evaluated. The peel was removed, and an electric blender was used to prepare the juice. The supernatant was separated from the sediment, and the extracted mandarin juice was filtered. TSS content was measured using a refractometer (Model: Milwaukee MA873 Digital, Milwaukee Co., United States), and the results were recorded in Brix (°Bx) units. Standard protocols were followed for the analysis.

2.3.4.2. The vitamin C content in mandarin fruit was measured using a laboratory-based method known as titration [35]. The procedure involved several steps. Firstly, the mandarin fruits were juiced, and the juice was filtered to remove any solid particles or pulp. The filtered juice was then diluted with distilled water to an appropriate concentration for the titration. In the titration process, a measured volume of the diluted mandarin juice was pipetted into a flask, and a few drops of 2,6-dichlorophenolindophenol (DCPIP) solution, acting as an indicator, were added. The solution was titrated with a standardized sodium thiosulfate solution until the color changed from blue to colorless, indicating the endpoint of the titration. The volume of the titrant used was recorded, and this, along with its known concentration, was used to calculate the amount of vitamin C in the mandarin juice. The result was expressed as mg of vitamin C per 100 g of mandarin fruit.

2.3.4.3. The percentage of reducing sugars in the fruit of mandarin trees was measured using method by Lane and Eynon [36]. Following this, a series of standard glucose solutions with known concentrations were prepared for calibration purposes. Subsequently, a measured volume of the diluted mandarin juice was pipetted into test tubes, and an equal volume of 3,5-dinitrosalicylic acid (DNS) reagent was added to each tube. The mixtures were heated in a water bath for a specified period to allow the reaction to occur. After cooling to room temperature, sodium carbonate solution was added to neutralize the mixtures and stabilize the color. The absorbance of each reaction mixture was then measured using a spectrophotometer (JENWAY 6305 UV/Visible spectrophotometer, U.K.) at a specific wavelength (540 nm). Utilizing the calibration curve generated from the standard glucose solutions, the concentration of reducing sugars in the mandarin juice was determined. Finally, the percentage of reducing sugars in the mandarin fruit was calculated based on the concentration obtained from the calibration curve.

2.3.4.4. The total acidity (%) in the fruit of mandarin trees was measured using a laboratory-based method called titration [35]. The filtered juice was diluted with distilled water to an appropriate concentration for the titration. In the titration process, a measured volume of the diluted mandarin juice was pipetted into a flask, and a few drops of phenolphthalein indicator solution were added, causing the solution to turn pink. The mandarin juice was then titrated with a standardized sodium hydroxide (NaOH) solution until the pink color just disappeared, indicating the endpoint of the titration. The volume of NaOH solution used was recorded. Using the volume of NaOH solution and its known concentration, the total acidity of the mandarin juice was calculated. The result was expressed as a percentage of citric acid equivalent acid per unit weight of the mandarin fruit.

2.3.4.5. The percentage of total sugars in the fruit of mandarin trees was measured using a laboratory-based method [35]. A series of standard glucose solutions with known concentrations were prepared for calibration purposes. Subsequently, a measured volume of the diluted mandarin juice was pipetted into test tubes, and an equal volume of Anthrone reagent was added to each tube. The mixtures were then heated in a water bath for a specified period to allow the reaction to occur. After cooling to room temperature, the absorbance of each reaction mixture was measured using a spectrophotometer (JENWAY 6305 UV/Visible spectrophotometer, U.K.) at a specific wavelength (625 nm). Utilizing the calibration curve generated from the standard glucose solutions, the concentration of total sugars in the mandarin juice was determined. Finally, the percentage of total sugars in the mandarin fruit was calculated based on the concentration obtained from the calibration curve.

2.5. Statistical analysis

The data collected from these experiments underwent statistical analysis using Statistix 8.1 software. One-way ANOVA was employed to assess the significance of the effects of various factors on growth parameters and yield data. Duncan's multiple range tests were employed to further explore and compare the means that showed significant differences. These tests allow for a detailed examination of the variations between treatment means with a 95% confidence level [37].

3. Results

3.1. Morphological trait

The results of the study indicate that the application of several antioxidants had a significant impact on leaf area of baldy mandarin trees (Figure 2). The trees treated with 1600 ppm of citric acid exhibited the largest leaf area (14.40 and 14.60 cm^2) in first and second seasons, respectively, while the trees treated under control gave the smallest leaf area (6.87 and 7.37 cm^2) in first and second seasons, respectively (Figure 2 A and B).

3.2. Yield traits

Trees subjected to 1600 ppm concentration of citric acid had the highest number of fruits per tree (492.00 and 580.00) in first and second seasons, respectively, while trees subjected to water (control) had the lowest number of fruits per tree (321.00 and 334.00) in first and second seasons, respectively (Figure 3 A and B). The trees treated with 1600 ppm concentration of citric acid gave the heaviest average fruit weight (139.03 and 145.00 g) in first and second seasons, respectively, but no significant difference with 800 ppm concentration of citric acid. While the trees treated with water (control) had the lowest average fruit weight (86.00 and 92.97 g) in first and second seasons, respectively (Figure 3 C and D). The trees treated with 1600 ppm concentration of citric acid gave the heaviest yield per tree (75.43 and 73.63 kg) in first and second seasons, respectively, while the trees treated with water (control) had the lightest yield per tree (36.20 and 33.57 kg) in first and second seasons, respectively (Figure 3 E and F). Control trees exhibited the highest percentage of pre-harvest fruit dropping (41.80 and 40.60 %) in comparison to other treatments (Figure 4 A and B), but no significant among 200 ppm concentration of salicylic acid (38.10 and 37.50 %) and 200 ppm concentration of thiamine (37.80 and 36.47 %) in first and second seasons, respectively. The trees treated with 1600 ppm concentration of citric acid gave the lowest percentage of pre-harvest fruit dropping (21.90 and 21.00 %) in first and second seasons, respectively (Figure 4 A and B).

3.3 Chemical composition fruit juice

The trees treated with 1600 ppm concentration of citric acid gave the highest total soluble solid content (13.73 and 13.70 °Bx) followed by 1600 ppm concentration of vitamin A+B (13.40 and 13.47 °Bx) in first and second seasons, respectively. While the trees treated with water (control) had the lowest total soluble solid content (11.33 and 11.20 °Bx) in first and second seasons, respectively (Figure 5 A and B). The trees treated with 1600 ppm concentration of citric acid gave the highest vitamin C of juice (45.47 and 45.03 mg 100 mL^{-1}) in first and second seasons, respectively. While the trees treated with water (control) had the lowest total soluble solid content (31.63 and 32.67 mg 100 mL^{-1}) in first and second seasons, respectively (Figure 5 C and D). The trees treated with 1600 ppm concentration of citric acid gave the highest percentage of reducing sugars (9.20 and 9.43%) followed by 800 ppm concentration of citric acid (9.03 and 9.10 %) and 400 ppm concentration of citric acid (8.57 and 8.77 %) in first and second seasons, respectively. While the trees treated with water (control) had the lowest total soluble solid content (3.20 and 3.03 %) in first and second seasons, respectively (Figure 6 A and B). Control trees exhibited the highest percentage of total acidity of juice (1.39 and 1.38 %) in comparison to other treatments (Figure 7 A and B), but no significant among 200 ppm concentration of salicylic acid (1.38 %) in first season and 200 ppm concentration of thiamine (1.37 %) in second seasons. The trees treated with 1600 ppm concentration of citric acid gave the lowest percentage of total acidity of juice (1.24 and 1.22 %) in first and second seasons, respectively, but no significant with 800 ppm concentration of citric acid (Figure 7 A and B). The trees treated with 1600 ppm concentration of citric acid gave the highest percentage of total sugars of juice (10.13 and 10.23 %) followed by 800 ppm of citric acid (9.67 and 9.77 %) without significant different in first and second seasons, respectively. While the trees treated with water (control) had the lowest total soluble solid content (7.03 and 6.87 %) in first and second seasons, respectively (Figure 7 C and D).The study demonstrated that the application of antioxidants significantly influenced the morphological, yield, and chemical traits of Baldy mandarin trees. Trees treated with 1600 ppm citric acid exhibited the largest leaf area, highest fruit yield, and heaviest fruit weight across two seasons compared to the control. Additionally, these trees had the lowest pre-harvest fruit drop, the highest total soluble solids, vitamin C content, reducing sugars, and total sugars, with the lowest juice acidity. In contrast, control trees had the smallest leaf area, lowest fruit yield, and poorest juice quality across all measured parameters (Figure 8).

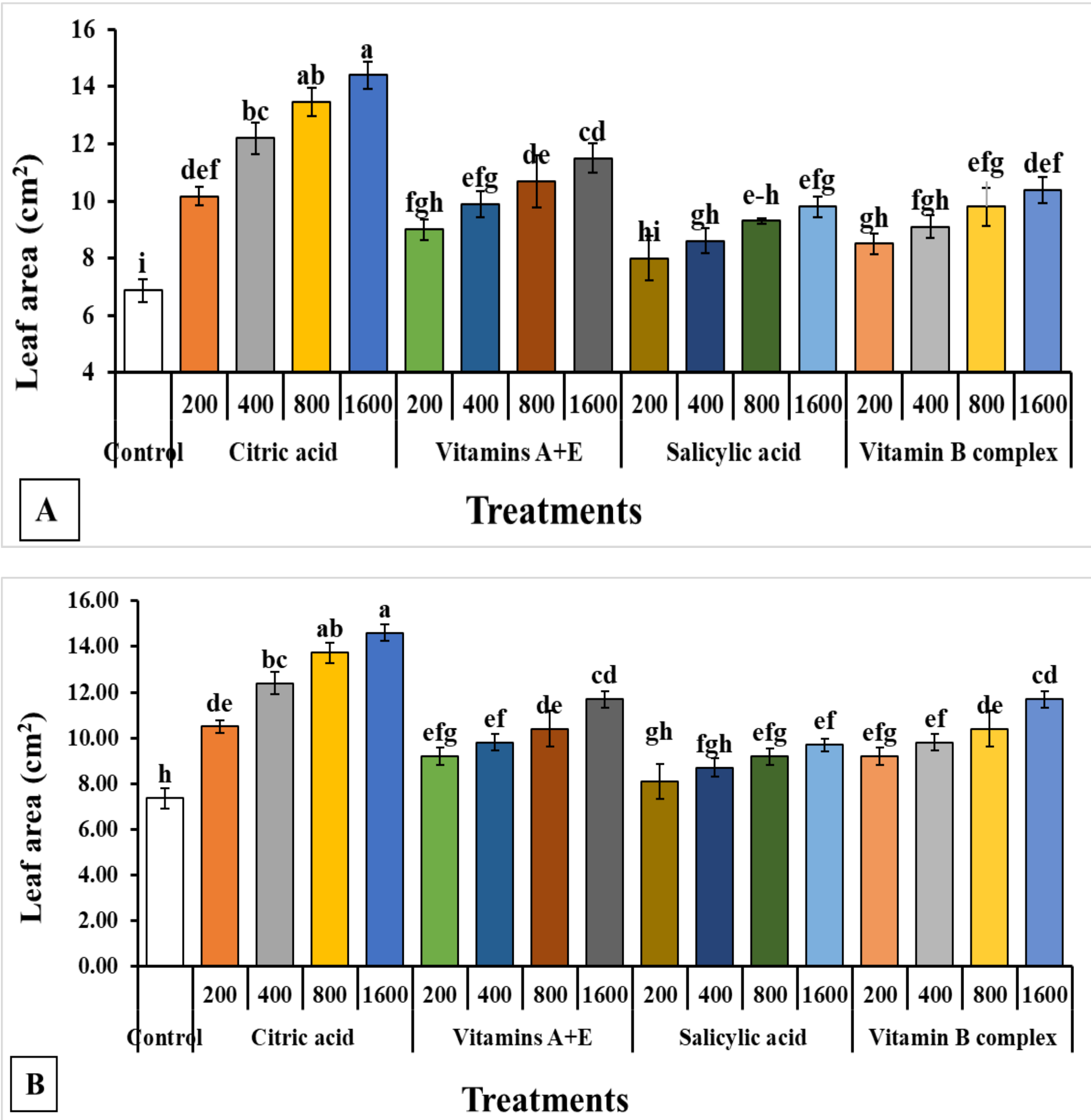
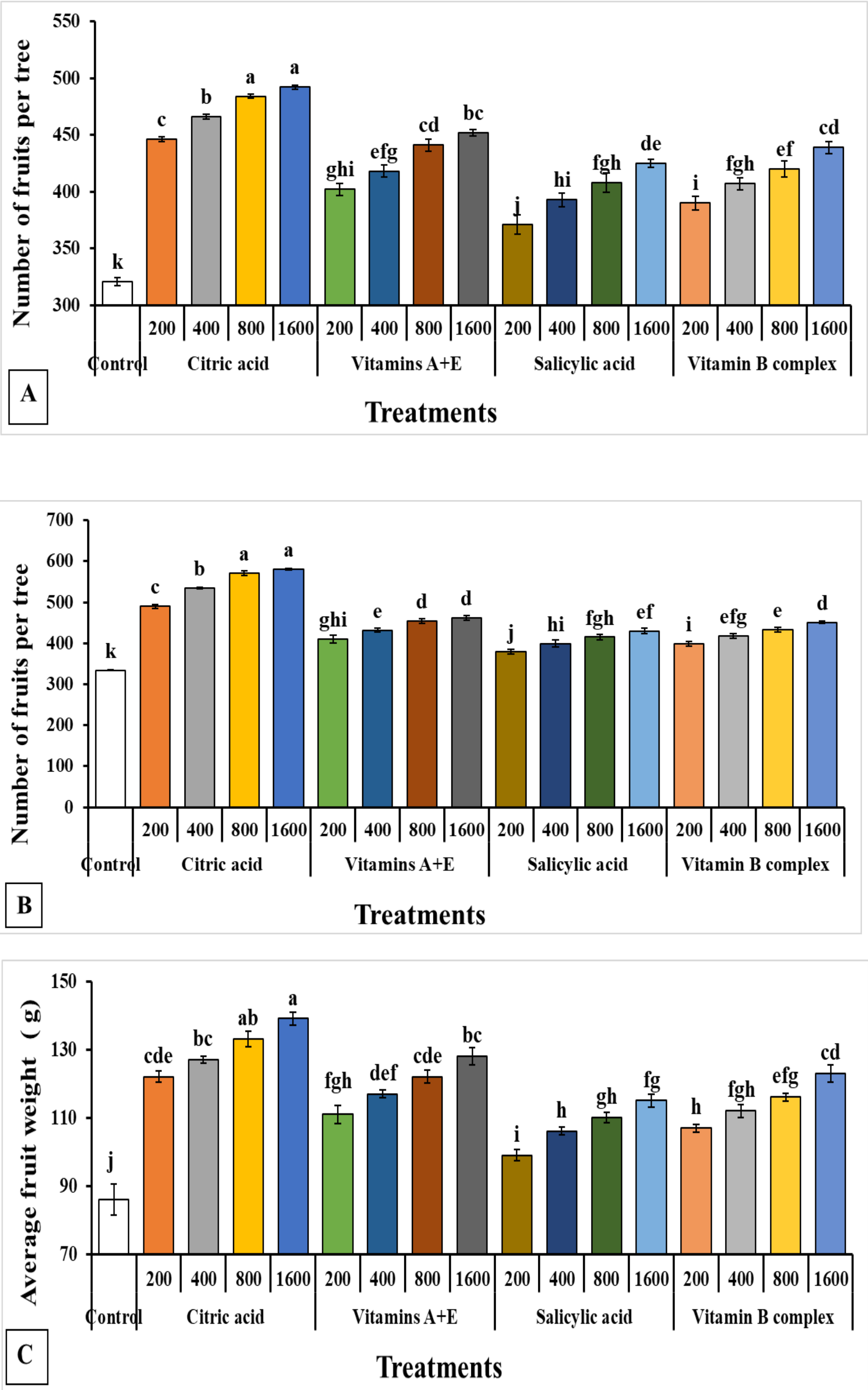


Figure 2. Impact of several antioxidants on leaf area of Baldy mandarin trees. Where (A) in the 2021 season and (B) in the 2022 season. Values are means ± standard deviations (SDs) of data obtained from three biological replicates (n=20). According to Duncan's multiple range test at a significance level of p=0.05, there were no significant differences among the means within each column that share the same letters. Where vitamin B complex = vitamin B1(thiamine)+B2(riboflavin)+B3(niacin).


A
Number of fruits per tree
550
500
450
400
350
300
k
c
b
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a
ghi
efg
cd
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j
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fgh
de
i
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ef
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200 400 800 1600 200 400 800 1600 200 400 800 1600 200 400 800 1600
Control
Citric acid
Vitamins A+E
Salicylic acid
Vitamin B complex
Treatments
B
Number of fruits per tree
700
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200
100
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Control
Citric acid
Vitamins A+E
Salicylic acid
Vitamin B complex
Treatments
C
Average fruit weight (g)
150
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70
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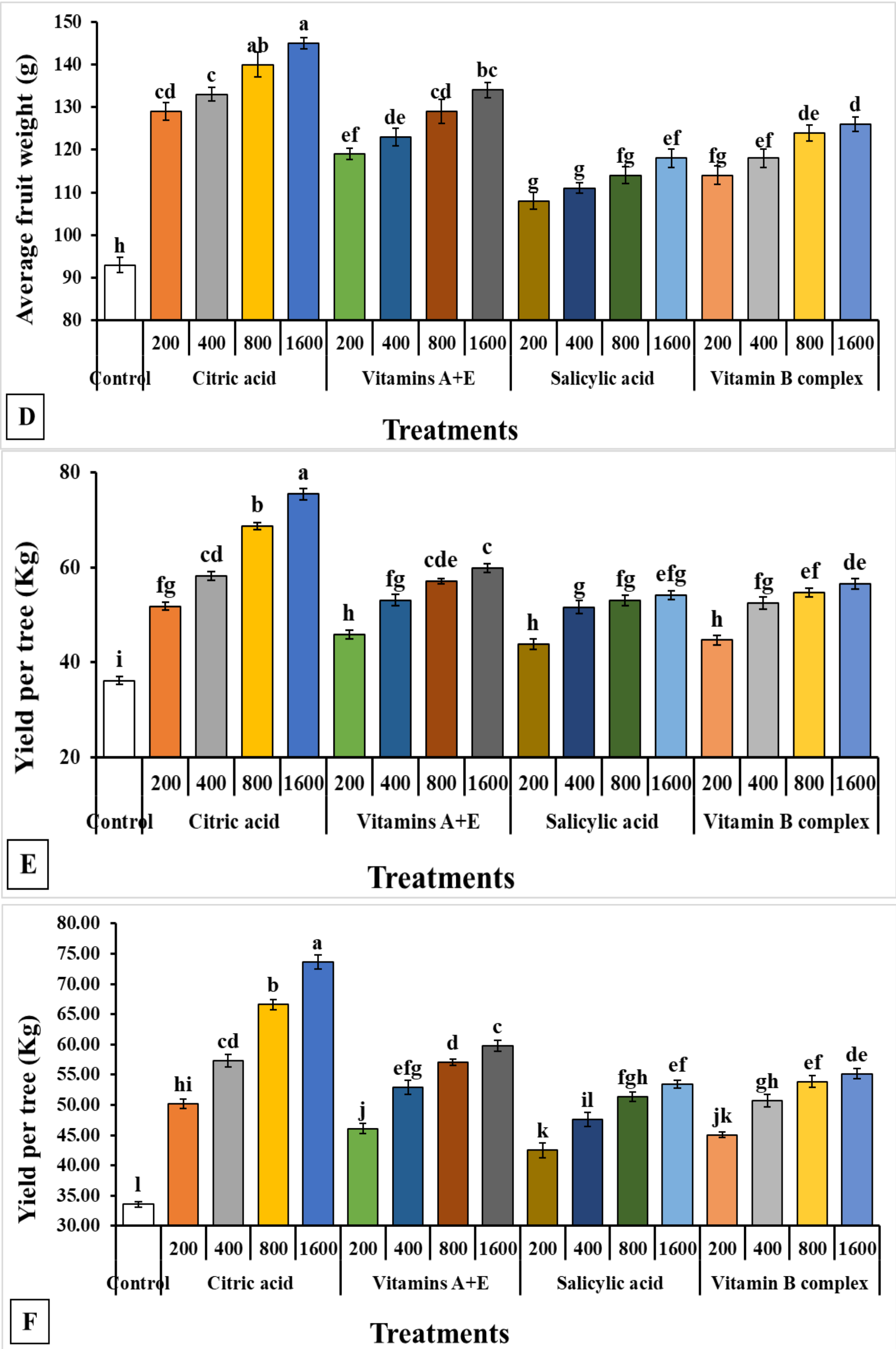
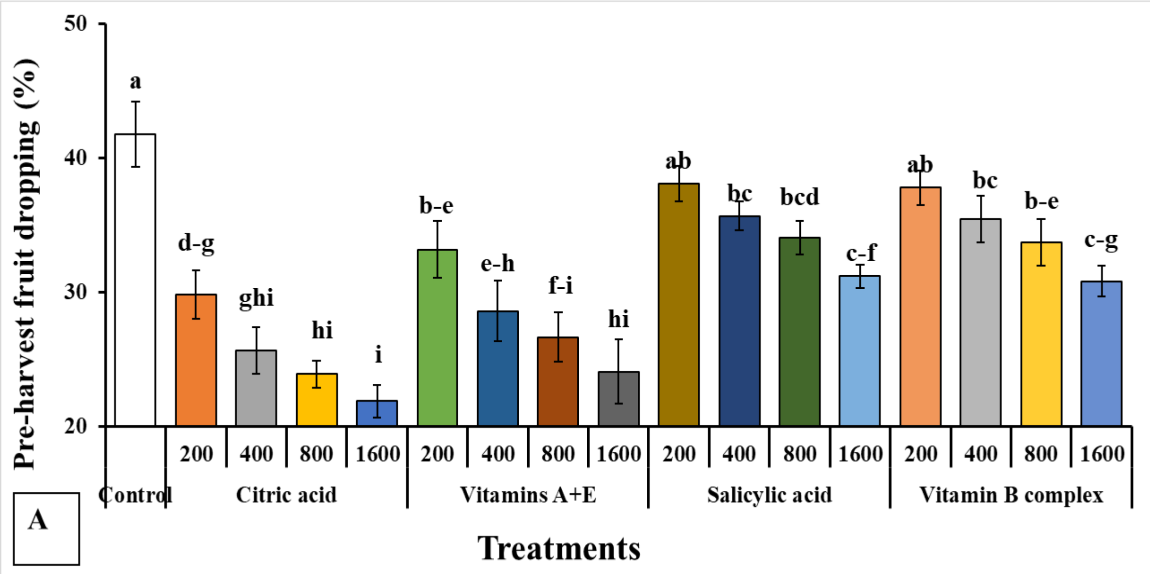


Figure 3. Impact of several antioxidants on number of fruits per tree and average fruit weight (g), and yield per tree (kg) of Baldy mandarin trees. Where (A) number of fruits per tree in the 2021 season; (B) number of fruits per tree in the 2022 season; (C) average fruit weight (g) in the 2021 season; (D) average fruit weight (g) in the 2022 season; (E) yield per tree (kg) in the 2021 season; (F) yield per tree (kg) in the 2022 season. Values are means ± standard deviations (SDs) of data obtained from three biological replicates (n=3). According to Duncan's multiple range test at a significance level of p=0.05, there were no significant differences among the means within each column that share the same letters. Where vitamin B complex = vitamin B1(thiamine) + B2 (riboflavin) + B3 (niacin).



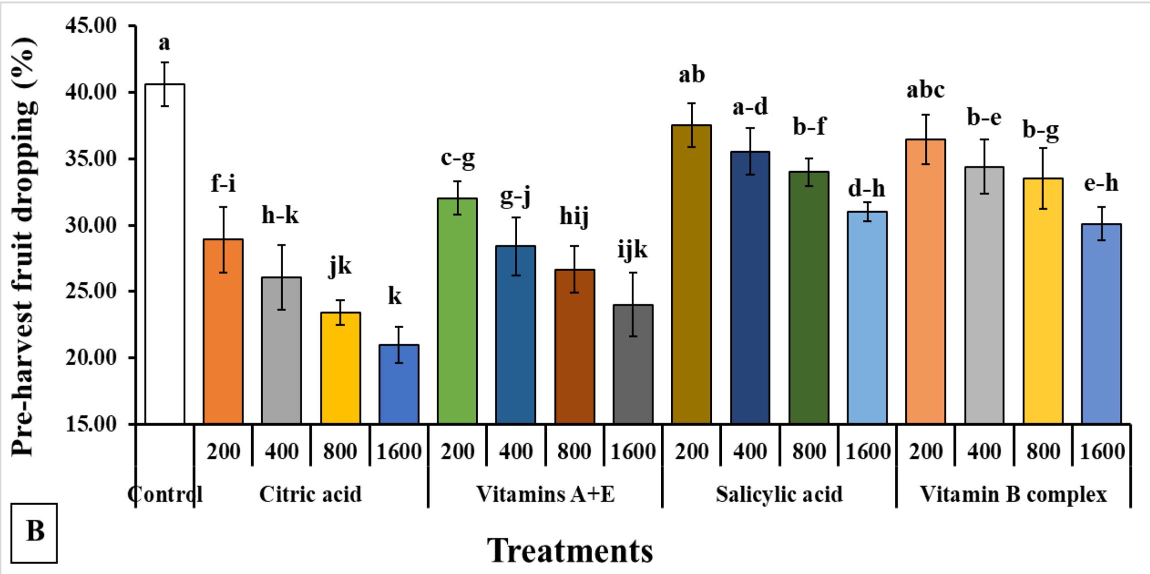


Figure 4. Impact of several antioxidants on pre-harvest fruit dropping of Baldy mandarin trees. Where (A) in the 2021 season and (B) in the 2022 season. Values are means ± standard deviations (SDs) of data obtained from three biological replicates (n=3). According to Duncan's multiple range test at a significance level of p=0.05, there were no significant differences among the means within each column that share the same letters. Where vitamin B complex = vitamin B1(thiamine) + B2 (riboflavin) + B3 (niacin).

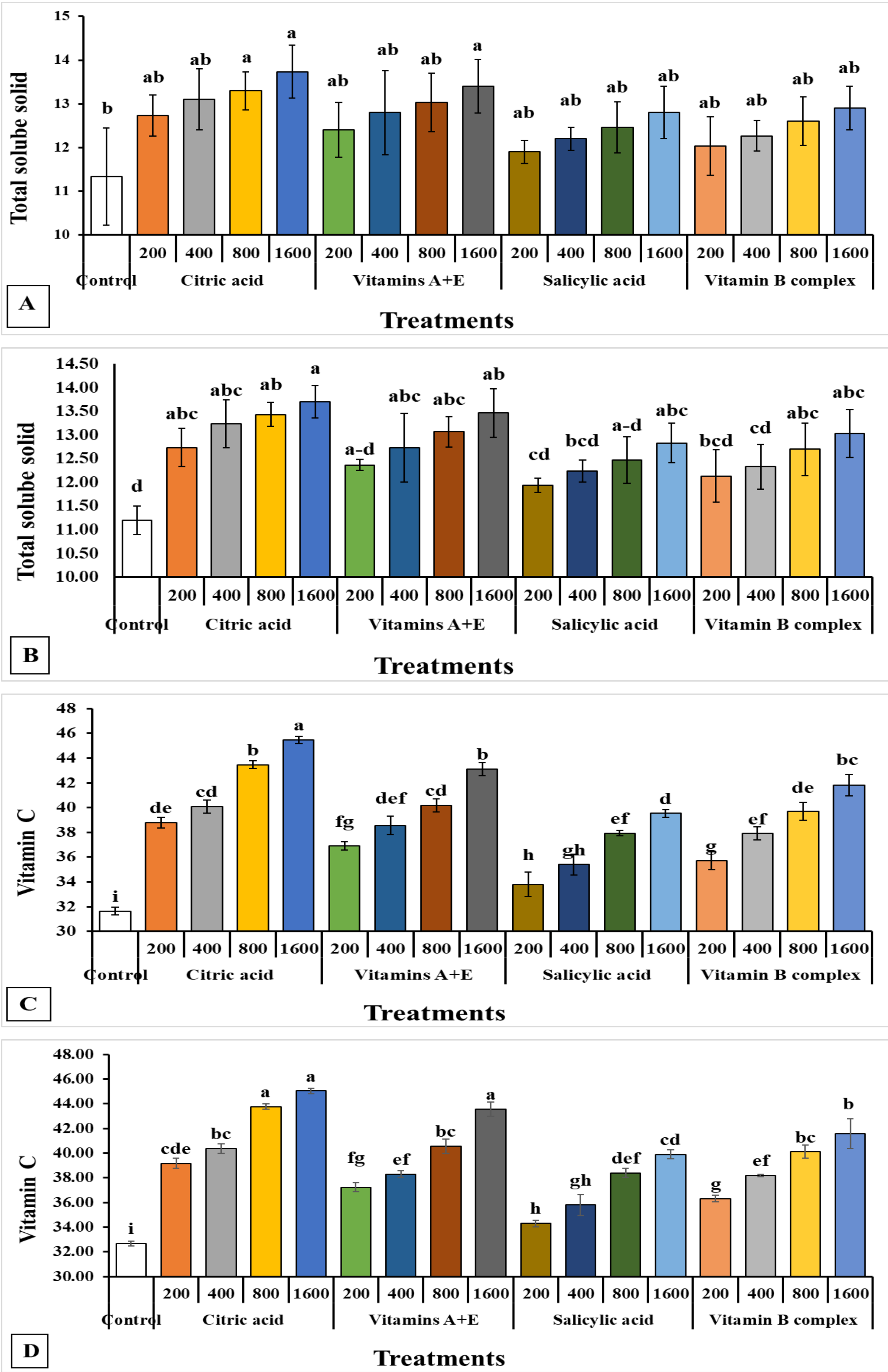


Figure 5. Impact of several antioxidants on total soluble solid and vitamin C of Baldy mandarin trees. Where (A) total soluble solid in the 2021 season; (B) total soluble solid in the 2022 season; (C) vitamin C in the 2021 season; (D) vitamin C in the 2022 season. Values are means ± standard deviations (SDs) of data obtained from three biological replicates (n=5). According to Duncan's multiple range test at a significance level of p=0.05, there were no significant differences among the means within each column that share the same letters. Where vitamin B complex = vitamin B1(thiamine)+B2(riboflavin)+B3(niacin).

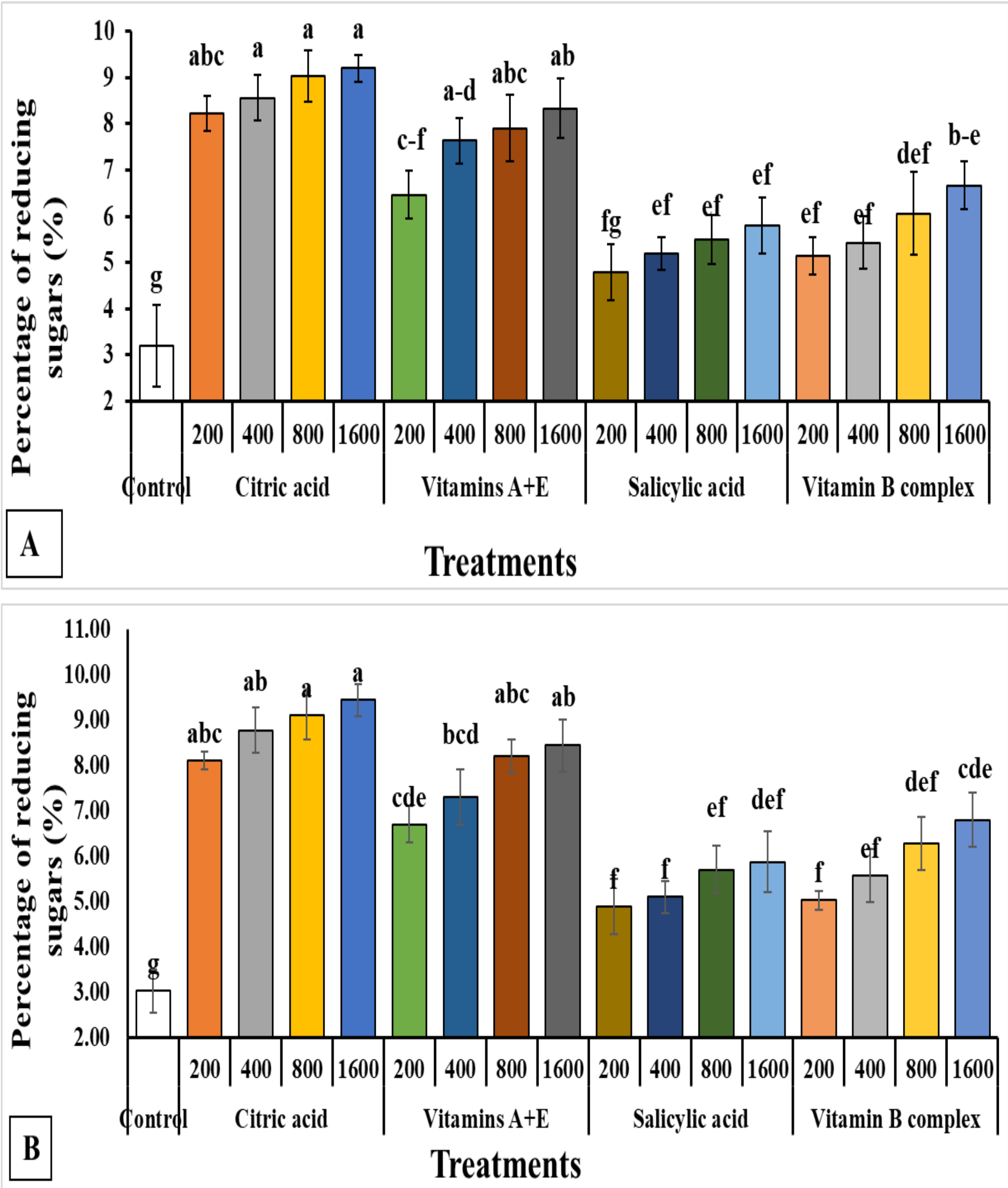


Figure 6. Impact of several antioxidants on percentage of reducing sugars (%) of Baldy mandarin trees. Where (A) in the 2021 season and (B) in the 2022 season. Values are means ± standard deviations (SDs) of data obtained from three biological replicates (n=3). According to Duncan's multiple range test at a significance level of p=0.05, there were no significant differences among the means within each column that share the same letters. Where vitamin B complex = vitamin B1(thiamine) +B2 (riboflavin) +B3 (niacin).

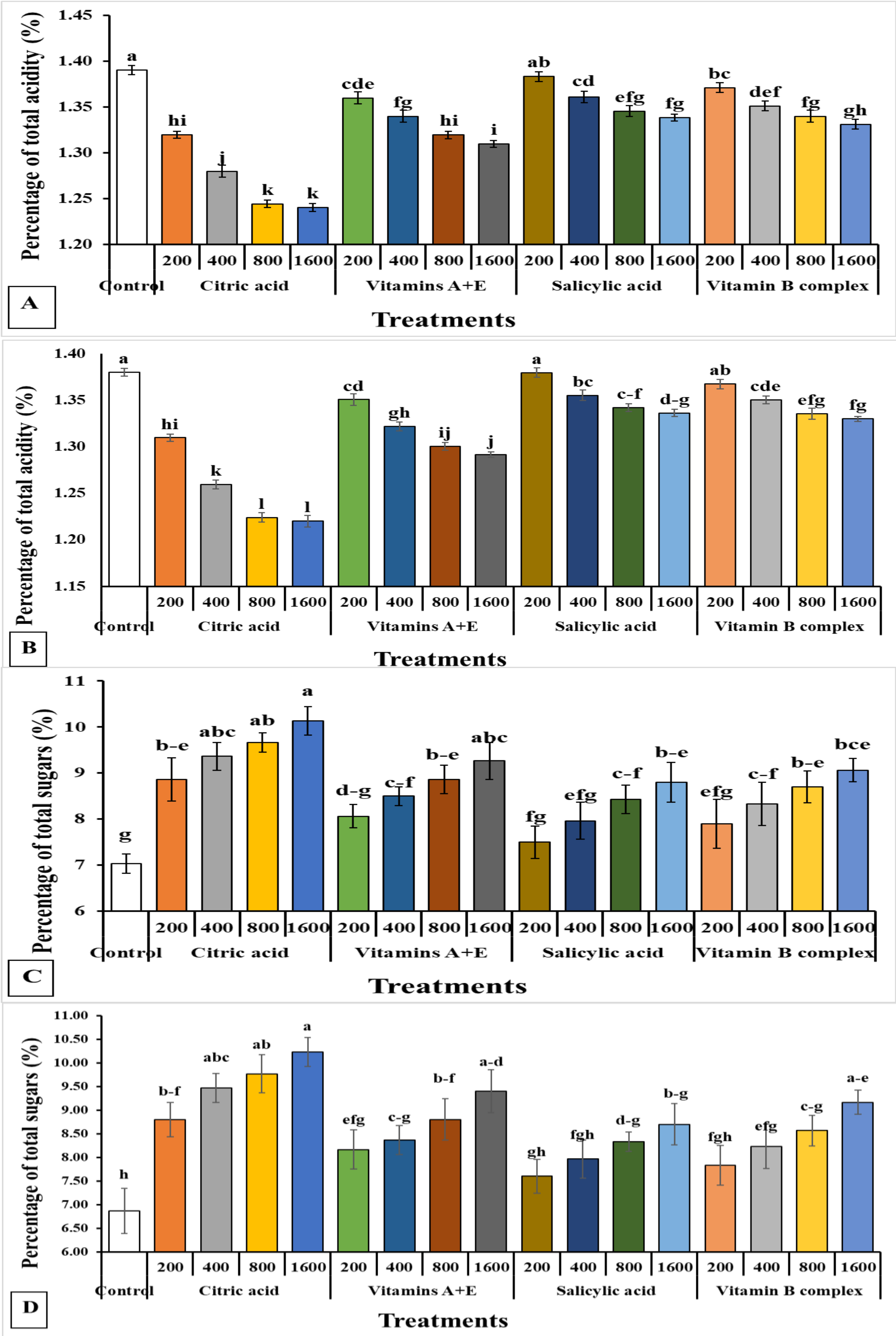


Figure 7. Impact of several antioxidants on percentage of total acidity (%) and percentage of total sugars (%) of Baldy mandarin trees. Where (A) percentage of total acidity (%) in the 2021 season; (B) percentage of total acidity (%) in the 2022 season; (C) percentage of total sugars (%) in the 2021 season; (D) percentage of total sugars (%) in the 2022 season. Values are means ± standard deviations (SDs) of data obtained from three biological replicates (n=5). According to Duncan's multiple range test at a significance level of p=0.05, there were no significant differences among the means within each column that share the same letters. Where vitamin B complex = vitamin B1(thiamine)+B2 (riboflavin) +B3 (niacin).

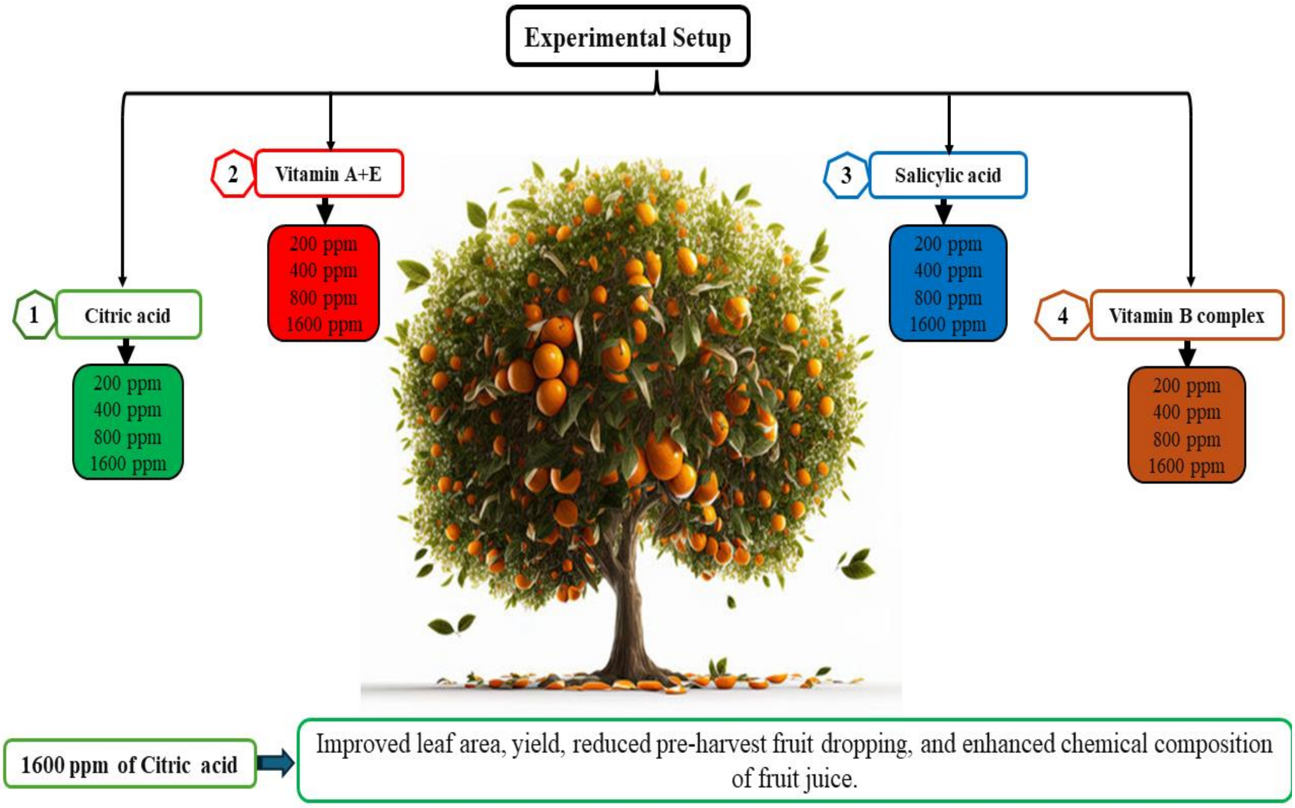


Figure 8. Illustration of the best antioxidants that significantly affected the morphological, productive, and chemical traits of Baldy mandarin trees.

4. Discussion

The findings of this study offer valuable insights into the effects of antioxidant treatments, particularly the application of citric acid, on multiple aspects of Baldy mandarin (*Citrus reticulata*) tree cultivation. These results hold significance for the field of horticulture and agricultural science.The substantial increase in leaf area observed in Baldy mandarin trees treated with 1600 ppm of citric acid during both the first and second seasons suggests that citric acid has a pronounced positive impact on leaf development and canopy growth. This enhancement in leaf area can be attributed to the role of citric acid as an antioxidant, potentially mitigating oxidative stress and promoting photosynthesis [8]. The increased leaf area may contribute to enhanced overall plant vigor and health, which can have far-reaching implications for orchard management [38]. Our results were consistent with those of Abdelmoniem, El-Shazly [6], who conducted an experiment on Navel orange trees. In his study, the maximum leaf area was achieved with the application of citric acid at concentrations of 500 and 1000 parts per million. The study's results demonstrate a clear association between citric acid application and improved yield traits in Baldy mandarin trees. Notably, trees treated with 1600 ppm citric acid consistently exhibited the highest number of fruits per tree and yield per tree, significantly surpassing the yield of the control group. This substantial increase in fruit production can be attributed to citric acid's role in promoting flower and fruit development, ultimately leading to a higher number of mature fruits. Moreover, the observed increase in average fruit weight in citric acid-treated trees further underscores the potential of citric acid to enhance fruit size and quality. Our results are in accordance with those of Abdelmoniem, El-Shazly [6], who conducted a study on Navel orange trees. In their research, the maximum number of fruits per tree was observed when citric acid was applied at a concentration of 500 ppm. However, our findings diverged from Abdelmoniem, El-Shazly [6]'s as the application of salicylic acid at 500 parts per million yielded results that were not significantly different from those obtained with citric acid at 500 ppm. Our results agreed with Mosa, Abd El-Megeed [11] that there was a expulsion relationship between increasing the concentration of citric acid and the total yield on pear trees, while they differed with Abdelmoniem, El-Shazly [6] as the relationship between increasing the concentration of citric acid and increasing the total yield was an inverse relationship on navel orange trees.A significant reduction in percentage pre-harvest fruit dropping was observed in trees treated with 1600 ppm citric acid. The reduction in percentage fruit dropping in citric acid-treated trees suggests that citric acid may help enhance fruit retention, leading to a decreased incidence of premature fruit drop [39]. Citric acid exerts its beneficial effects on fruit set, fruit retention, the number of fruit per tree, and yield primarily by its significant role in plant metabolism [40]. Additionally, it serves as a chelator for these free radicals, safeguarding plants against damage and potentially extending the shelf life of plant cells while enhancing growth attributes [41]. Furthermore, citric acid demonstrates auxinic properties and positively impacts flowering and fruiting, serving as a natural alternative to synthetic auxins and other chemicals for enhancing the growth and fruiting of fruit trees [42], this positive influence is reflected in the improved flowering and fruiting of trees. The chemical analysis of fruit juice revealed that trees treated with 1600 ppm citric acid consistently yielded juice with higher total soluble solid content, percentage of reducing sugars, and vitamin C levels. These findings indicate that citric acid application significantly influences fruit composition, leading to improved fruit sweetness, maturity, and nutritional quality [43]. The higher total soluble solid content is indicative of increased sugar content in the fruit, which can enhance fruit flavor and overall fruit quality [44]. Sugars accumulate throughout fruit development and ripening, but they tend to remain relatively stable or decrease during the postharvest phase [45]. The breakdown of sucrose starts with its conversion into hexose, followed by its utilization in the glycolysis pathway as a source of respiration and/or its transformation into other carbohydrates [45]. Citric acid is the primary organic acid in citrus fruits, and it increases as fruits develop but decreases during the late-ripening and postharvest stages [46]. The decrease in citric acid content is primarily due to its utilization as a source of respiration or in the synthesis of amino acids [46]. Therefore, when exogenous citric acid is applied to trees, it compensates for the natural breakdown of citric acid and, as a result, increases the concentration of sugar in the juice.

The outcomes of our study are consistent with prior research findings. Mansour, Ahmed [47] discovered that the application of citric acid to pear trees resulted in improved fruit production, increased fruit weight, enhanced fruit firmness, higher levels of total soluble solids, and greater total sugar content, while decreasing fruit acidity. Similarly, [48] found that citric acid had positive effects on shoot length, the ratio of leaves to shoots, leaf area index, total soluble solids percentage, vitamin C content, and fruit firmness in apricot cv. Canino. Calcium (Ca) has been demonstrated to increase the uptake of essential elements and mitigate the adverse effects of lead (Pb) exposure under environmental stress conditions, thus improving photosynthetic rate [49], photosynthetic pigment production [50], and plant growth [51]. Osama, Amro [39] observed that the application of citric acid at 400 and 800 ppm to 'Keitt' mango trees led to increased percentages of fruit set and retention, greater fruit numbers, enhanced fruit production, and improved physical attributes such as fruit and peel weight, as well as pulp/fruit ratio, compared to the control. Additionally, citric acid led to improved chemical characteristics of the fruit, including higher levels of total soluble solids and sugars, while reducing fruit acidity when compared to the control. In the context of 'Washington' navel orange trees, the application of citric acid at a concentration of 1000 ppm led to notable improvements. Specifically, it resulted in an increase in the total chlorophyll content in the leaves, greater numbers of shoots, increased shoot length and thickness, higher leaf numbers per shoot, and an expansion of leaf area, as reported by El-Badawy, El-Gioushy [52]. Similarly, Mohamed [53], observed positive effects of foliar application of citric acid at 1000 ppm on grapevines. This treatment was associated with increased yields, a higher number of clusters per vine, greater cluster weight, increased cluster length and width, enhanced berry weight, longer and wider berries, higher content of reducing sugars in the berries, elevated anthocyanin levels, and greater total soluble solids in the grapes. Additionally, it resulted in reduced acidity in the berries over two consecutive seasons when compared to the control group. Overall, our study aligns with prior research and highlights the potential of citric acid as a valuable tool for enhancing fruit tree cultivation, yield, and fruit quality. These findings contribute to our understanding of the benefits of antioxidant treatments in agriculture and open doors for further exploration of citric acid's applications in fruit tree management.

5. Conclusions

In conclusion, the results of this study underscore the potential benefits of utilizing citric acid as an antioxidant in Baldy mandarin tree cultivation. Citric acid application exhibited significant positive effects on morphological traits, yield traits, and the chemical composition of fruit juice. The enhanced leaf area, increased fruit production, reduced fruit dropping, and improved fruit quality all point to the practical implications of using citric acid as a valuable tool for enhancing orchard management and optimizing fruit production in Baldy mandarin cultivation. These findings contribute to our understanding of the role of antioxidants in citrus agriculture and highlight citric acid's potential as an effective strategy for improving both yield and fruit quality. Further research into the mechanisms underlying these effects and the optimization of application protocols can provide valuable insights for citrus growers seeking to enhance their orchard's productivity and fruit quality.

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