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Effects of Microbial Fertilizer Applications on Yield, Fruit Quality and Leaf Characteristics of Red Chief Apple Variety

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ABSTRACT

The research was carried out on Red Chief apple variety grafted onto MM106 rootstock in the apple orchard established in 2011 at Yozgat Bozok University in 2022-2023. The study was established to determine the effects of Control (100% fertilizer), 50% reduced fertilizer, 100% reduced fertilizer (without fertilizer), and combinations of fertilizers with microbial fertilizer containing bacteria on fruit yield and quality and vegetative growth. The highest fruit weight was obtained from Control (100% fertilizer dose) + Bacteria application (170.57 g) and 50% reduced fertilizer dose + Bacteria (164.30 g) applications. The lowest fruit weight was determined from a 100% reduced fertilizer dose (without fertilizer) (103.18 g). Total soluble solids were measured with a 20.30% Brix value in the Control (%100 fertilizer) + bacteria and a 19.90% brix value in 50% reduced + bacteria applications. The highest titratable acidity was determined with 0.59% in Control (%100 fertilizer) application. The fruits from the trees of each replication were collected separately and the highest tree yield values were obtained from Control (%100 fertilizer) + bacteria (22.35 kg/tree) and Control (%100 fertilizer) applications (19.63 kg/tree). When the effects of the applications on shoot length were examined, the highest values were determined in Control (%100 fertilizer), Control (%100 fertilizer) + bacteria, and 50% reduced fertilizer + bacteria applications. The highest amount of nitrogen (N) in the leaf was determined in Control (100% fertilizer), Control (100% fertilizer) + bacteria, and 50% reduced + bacteria applications. The highest amount of P was obtained from Control (100% fertilizer) and Control (100% fertilizer) + bacteria applications. The highest amount of K was measured in 50% reduced fertilizer application. It was concluded that microbial fertilizers containing plant growth-promoting bacteria can be used in apples. Replication of the study in different climatic and geographical conditions may increase the extensibility of the results.

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ÖZET

Araştırma, 2022-2023 yıllarında Yozgat Bozok Üniversitesi' nde 2011 yılında kurulmuş olan elma bahçesinde yer alan MM106 anacına aşılı Red Chief elma çeşidi üzerinde yürütülmüştür. Çalışma, kontrol (%100 gübre), %50 azaltılmış gübre, %100 azaltılmış gübre (gübresiz) ve gübrelerin bakteri içeren mikrobiyal gübre ile kombinasyonları kullanılarak meyve verim ve kalitesi ile vejetatif büyüme üzerine etkilerini belirlemek amacıyla kurulmuştur. Meyve ağırlığı en yüksek kontrol (%100 gübre dozu)+bakteri uygulaması (170.57 g) ile %50 azaltılmış gübre dozu+bakteri (164.30 g) uygulamalarından elde edilmiştir. En düşük meyve ağırlığı ise %100 azaltılmış gübre dozundan (gübresiz) (103.18 g) tespit edilmiştir. Meyve eti sertliği (kg/cm2), en yüksek kontrol (%100 gübre), %50 azaltılmış gübre ve %100 azaltılmış (gübresiz) uygulamalarında ölçülmüştür. Suda çözünebilir kuru madde içeriği %20.30 Brix değeri ile kontrol (%100 gübre) + bakteri ve %19,90

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
Red Chief elma çeşidi
Mikrobiyal gübre
Kimyasal gübre
Yozgat

Brix değeri ile %50 azaltılmış + bakteri uygulamalarında ölçülmüştür. Titre edilebilir asit miktarı ise en yüksek %0.59 ile kontrol (%100 gübre) uygulamasında belirlenmiştir. Her tekerrüre ait ağaçlardaki meyveler ayrı ayrı toplanarak elde edilen ağaç başı verim değerleri en yüksek kontrol (%100 gübre)+bakteri (22.35 kg/ağaç) ve kontrol (%100 gübre) uygulamalarından (19.63 kg/ağaç) elde edilmiştir. Uygulamaların sürgün boyu üzerine etkisi incelendiğinde en yüksek değerler kontrol (%100 gübre), kontrol (%100 gübre)+ bakteri ve %50 azaltılmış gübre+bakteri uygulamalarında tespit edilmiştir. Yapraktaki azot (N) miktarı en yüksek kontrol (%100 gübre), kontrol (%100 gübre) + bakteri ve %50 azaltılmış + bakteri uygulamalarında tespit edilmiştir. P miktarı en yüksek kontrol (%100 gübre) ve kontrol (%100 gübre) + bakteri uygulamasından elde edilmiştir. K miktarı en yüksek %50 azaltılmış gübre uygulamasında ölçülmüştür. Bitki gelişimini teşvik eden bakterileri içeren mikrobiyal gübrelerin elmalarda kullanılabileceği sonucuna varılmıştır. Çalışmanın elma yetiştirilen farklı iklim ve bölgelerde tekrarlanması sonuçların yaygınlaştırılabilirliğini artıracaktır.

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INTRODUCTION

The apple, a plant of cold temperate climates generally between 30°-50° latitude in the world, belongs to the genus Malus within the subfamily Pomoideae of the family Rosaceae. The number of species included in the genus Malus varies from author to author. Apples are grown on all continents of the world, but the Asian continent accounts for 66.28% of world apple production, with 63,522,168 tonnes. The European continent, with 18,794,929 tonnes of production, is in second place after the Asian continent, accounting for 19.61% of world production. America is in third place with 8,857,607 tonnes, and Africa is in fourth place with 3,785,189 tonnes. The lowest production was in Oceania with 876,071 tonnes (FAO, 2024). According to the FAO, world apple production in 2022 will be 95,835,964 tonnes. China ranks first in production with 47,571,800 tonnes, followed by Turkey (4,817,500 tones), America (4,429,330 tonnes), Poland (4,264,700 tonnes), India (2,589,000 tonnes), Russia (2,379,900 tonnes), and Italy (2,256,240 tonnes).

The growth and development of a plant vary according to climate and region. Phenological observations of plants include dates such as bud swelling, bud burst, first flowering, full flowering, end of flowering, harvest date, and leaf fall date (Rana et al., 2023). As a result of these observations, decisions are made on issues such as agricultural techniques and planning, cultural practices, selection of suitable varieties, and pollinator species in the geographical region where the plant is located. Chemical fertilizers used in excessive amounts and unknowingly in cultivation cause a decrease in product quality and environmental pollution (Güneş et al., 2012; Shuqin & Fang, 2018; Rahman & Zhang, 2018). The use of fertilizers to increase fruit yield and quality in our country and in the world pollutes the environment to a great extent. For this reason, in the studies conducted, researchers are trying applications that increase yield and quality by reducing the use of chemical fertilizers and minimizing the damage to the environment (Ertürk, 2015; Tian et al., 2022).

Organisms living in the soil have a very important place in terms of soil fertility. Soil organisms consist of soil flora and soil fauna. Fungi, bacteria, actinomycetes, and algae are included in the soil flora. Protozoa, nematodes, earthworms, and other animals are included in the soil fauna. They have different functions in increasing soil fertility. Bacteria play a role in the breakdown of organic matter and the nitrogen cycle (Karaçal & Tüfenkçi, 2010; Wang et al., 2020). As a result of many studies conducted on different applications and costs that do not harm nature and reduce the use of chemical fertilizers, the use of microorganisms stands out. Bacteria found in the soil that promote plant growth are called biofertilizers. It has been determined that bacteria provide nitrogen use, phosphorus solubility, plant hormone production, and water use efficiency as a result of nitrogen fixation, increase the uptake of nutrient elements by the plant, and enzymatically reduce the level of ethylene and positively increase plant growth (Ekici et al., 2015). The use of microorganisms and their new combinations as microbial fertilizers has important consequences in agriculture (Kucharski et al., 1996; Vessey, 2003). Microbial fertilizers are used to increase plant growth and development and productivity by increasing nutrient uptake, to improve soil structure and productivity, to help break down organic residues, and to provide resistance to diseases and pests (Çakmakçı, 2005).

With the application of reduced doses of chemical fertilizers together with microbial fertilizers, it is aimed to increase the efficiency and fertilizer use efficiency by using natural, biological, and man-made plant nutrient sources without harming the environment (Gruhn et al., 2000). Another negative effect of excessive fertilizer use is that it increases the unit cost of agricultural products. The main goal of the enterprises is to obtain a profit that will ensure the continuity of the enterprise in agricultural activities. In order to obtain high yield and quality from fruit species such as apple, they need macro elements such as N, P, and K from the soil. For this reason, producers use large amounts of fertilizer in apple cultivation. There are variable costs such as pruning, soil processing, irrigation, fertilization, spraying, thinning, harvesting, transportation, insurance, and revolving fund interest in the cultivation of fruit species such as apple. In a study conducted on apples in Isparta and Karaman, it was determined that these costs constituted approximately 70% of the total costs. Among these, it has been determined that fertilization costs constitute the third largest cost item with a rate of approximately 8-10% after spraying (20%) and harvesting (17%) (Bayav & Karli, 2020).

In today's modern fruit growing and with the existing varieties, it is not possible to grow without using chemical fertilizers. However, the use of chemical fertilizers by plants is mostly less than 50%, and this rate even decreases to 10% in P. Therefore, at least half of the applied fertilizer remains in the soil, mixes with groundwater, or is lost to the atmosphere as gas. Accordingly, continuous use of chemical fertilizers has many negative effects. Continuous use of chemical fertilizers negatively affects the physical, chemical, and biological properties of the soil and reduces soil fertility. It plays a significant role in environmental pollution. In addition, it contributes to global climate change by increasing the levels of greenhouse gases in the atmosphere. In addition, as soil conditions deteriorate, growers tend to use more fertilizer to achieve the desired yield, quality, and growth, exacerbating the negative effects. This situation leads to an increase in negative effects in a vicious circle. In addition to having such negative effects, increased fertilizer use also increases the unit cost of the product and reduces the producer's profitability. Therefore, developing applications that will increase the usability of fertilizers by plants is very important in reducing these negativities. In order to ensure sufficient vegetative growth and high yield and quality in apple cultivation, a very intensive fertilizer application is made. Accordingly, serious contributions are made to the negativities mentioned due to intensive fertilizer use during apple cultivation (Abebe et al., 2022).

It has been reported that plant growth-promoting rhizobacteria promote vegetative development in different plant species such as apple, apricot, barley, lettuce and tomato, blueberry, hazelnut, grape, cherry (Bassil et al, 1991; Rodriguez and Fraga, 1999; De Silva et al., 2000; Eşitken et al., 2003; Köse et al., 2003; Eşitken et al., 2006; Aslantaş et al., 2007; Pırlak et al., 2007; Ekici et al., 2015). Parıltı (2018) observed that root application of Bacillus subtilis OSU-142 and Bacillus megaterium M-3 bacterial strains to some apple seedlings grafted on MM106 rootstock resulted in a significant increase in vegetative development of the seedlings. Kotan et al. (2021) reported that in their study using microbial fertilizer containing Bacillus subtilis in the cultivation of apple seedlings, the use of bacteria increased shoot length by 65.09% and shoot diameter by 22.66% compared to the control. Yaman et al. (2022) found the shoot length as 34.45 cm and 26.50% positive bacterial effect in the Red Chief / M9 combination, and as 31.88 cm and 25.53% positive bacterial effect in the Red Chief / MM106 combination.

The aim of this research is to reduce the amount of fertilizer used by applying microbial fertilizers without reducing plant growth, development, fruit yield, and quality, thus reducing the negative impact on the environment, human health, and the country's economy.

MATERIAL and METHOD

The research was carried out on the Red Chief apple cultivar grafted onto the MM106 rootstock in the apple orchard established in 2011 at Yozgat Bozok University, Gedikhasanlı Bilal Şahin Agricultural Application and Research Center (TUAM). The variety is of American origin and has weak, semi-upright trees. Its medium-sized fruits are sweet and juicy. The color of the fruit is creamy white. The outer shell color is bright red on a yellow-green base. The harvest is done in the last week of September (Anonymous, 2023a).

Yozgat is located between 34º05' - 36º10' east meridians and 38º40' - 40º18' north parallels. Its altitude above sea level is 1300 meters, and it ranks 15th among 81 provinces in terms of land width, with its 1.412.300 hectares of land. The altitude decreases from east to west of the province (Anonymous, 2023b).

The climate characteristics of the orchard located in Songun district of Yozgat province, where the research was conducted, show the typical continental climate characteristics of the Central Anatolia Region. When the meteorological data covering the years 2022-2023 of the region, where summers are hot and dry, winters are cold and rainy, are examined, it is seen that the highest monthly average temperature was 23.7˚C in August 2022, and the lowest was -2.3°C in January 2022. In 2023, the highest average temperature was measured as 23.6°C in August, and the lowest was -0.7°C in February. The average relative humidity in 2022 was 64.28%; and 64.53% in 2023. In 2022, the highest monthly average temperature was measured as 38.1°C in July, and the lowest was -21.2°C in January 2022. The lowest temperature value recorded in 2023 was recorded in February with -12.5°C, and the highest monthly average temperature value was recorded in August with 36.9°C (Figure 1 and Figure 2).

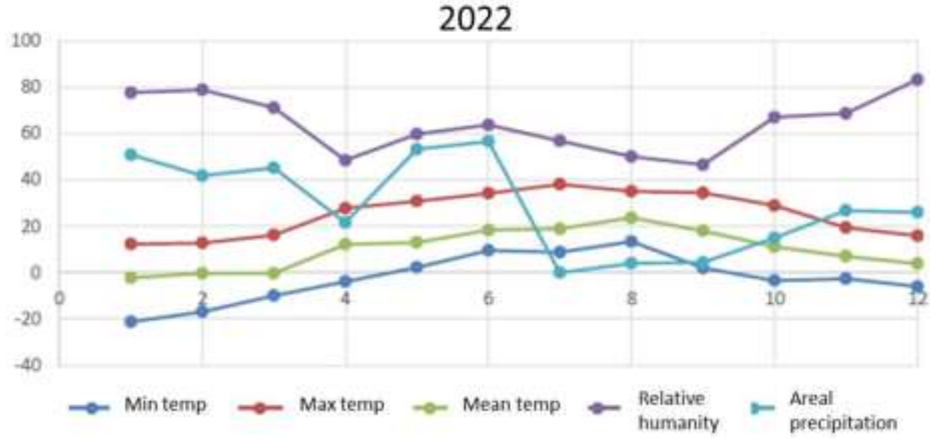

Figure 1. Meteorological Data of the Research Area for 2022
Şekil 1. Araştırma Alanının 2022 Yılı Meteoroloji Verileri

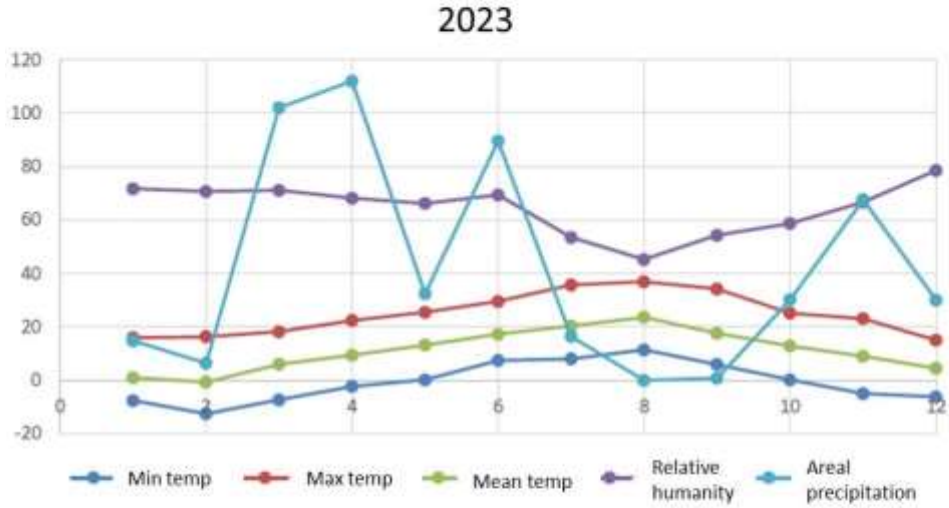

Figure 2. Meteorological Data of the Research Area for 2023
Şekil 2. Araştırma Alanının 2023 Yılı Meteoroloji Verileri

As a result of the analysis of soil samples taken from the field where the research was carried out, it was determined that the average pH was 7.8, the amount of lime was 16%, and the organic matter was below 1%. It was determined that the soils were poor or very poor in terms of organic matter, total nitrogen, available phosphorus, iron, zinc, and magnesium, and were generally clayey, slightly alkaline, and contained high levels of lime and Ca (Table 1).

In the experiment, the amounts of N, P, and K fertilizer used by the Agricultural Application and Research Centre where the study is carried out were used as a control, and in addition 50% and 100% reduced doses of these amounts were applied to the crown projection areas of the trees in April. A commercial preparation was used, Lactic acid bacteria (*Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*), Phototrophic Bacteria (*Rhodopseudomonas palustris*), and *Bacillus subtilis* containing as bacterial material. This product is ready for use in agriculture and is applied to the soil.

The first application of the bacterial solution to be used in the study was made in April, when the temperature reached the point at which the roots would start to work actively. In addition, the application was repeated in June and July. In the second year of the experiment, the applications were repeated in the same period. Bacterial applications were applied as a solution to the crown projection of the trees, 1000 ml into the root rhizosphere from 8 different points opened with pipes (Figure 3).

Table 1. Soil Properties of the Experimental Area
Çizelge 1. Deneme Alanının Toprak Özellikleri

Analysis- Unit / Depth (cm)	Structure	pH	Salinity-EC %	Lime %	Organic Matter %	Total Nitrogen %	P_2O_5 (kg/da)	K_2O (kg/da)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)
0-20 Result	66.0	7.73	0.02	10.41	0.83	0.04	1.96	41.65	1.06	1.16	0.59	2.10	7.92	155.1	19.10
	Clayey Loam	Slightly Alkaline	Salt-Free	Medium	Very Little	Very Poor	Very Little	Excessive	Insufficient	Sufficient	Sufficient	Sufficient	Excessive	Little	
20-40 Result	69.3	7.80	0.02	16.82	0.57	0.03	0.97	24.3	1.02	0.10	0.32	2.12	7.55	128.4	20.90
	Clayey Loam	Slightly Alkaline	Salt-Free	Excessive	Very Little	Very Poor	Very Little	Medium	Insufficient	Very Little	Sufficient	Sufficient	Excessive	Little	
40-60 Result	66.0	7.81	0.02	20.18	0.66	0.03	0.82	20.15	1.13	0.12	0.22	2.39	7.39	130.3	20.20
	Clayey Loam	Slightly Alkaline	Salt-Free	Excessive	Very Little	Very Poor	Very Little	Medium	Insufficient	Very Little	Sufficient	Sufficient	Excessive	Little	

Experiment applications were presented below:

1. Control (100% fertilizer level, farm application, 8 kg/da N, 6 kg/da P_2O_5, 10 kg/da K_2O)
2. 50% reduced (4 kg/da N, 3 kg/da P_2O_5, 5 kg/da K_2O)
3. 100% reduced (without fertilizer)
4. Control (100% fertilizer level, farm application, 8 kg/da N, 6 kg/da P_2O_5, 10 kg/da K_2O) + bacteria application
5. 50% reduced (4 kg/da N, 3 kg/da P_2O_5, 5 kg/da K_2O) + bacteria application
6. 100% reduced (without fertilizer) + bacteria application

In the study, observations of phenological periods (bud swelling, bud burst, first flowering, full bloom, end of flowering, harvest date, leaf fall date) were taken. For pomological analyses, measurements and analyses were carried out using 20 randomly selected fruits from each replication. The measurements of fruit weight (g), fruit width (mm), length (mm), yield ($kg.tree^{-1}$), total soluble solids (brix, %), total acidity (%), fruit firmness ($kg.cm^{-2}$), and fruit skin color obtained from the applications in the study were determined.

In order to determine the vegetative development of the trees, samples were taken from the middle part of the shoots in August, from the leaves that had reached full size, in three replications, with 20 leaves in each replication. In order to determine the shoot characteristics, shoot characteristics were measured in October, when the trees entered dormancy. Leaf chlorophyll content readings (measured using the Konica Minolta SPAD-502 Plus Brand Chlorophyll Meter, SPAD), leaf anthocyanin amount (measured by (Opti Science ACM–200 Plus Anthocyanin Meter, ACI), leaf area determination (determined by ADC Bio Scientific Area Meter AM300 device, cm^2), Leaf Fresh Weight (g), Leaf Dry Weight (g) and Specific leaf weight ($g.cm^{-2}$) were measured.

Figure 3. Application of bacterial solution
Şekil 3. Bakteri solüsyonunun uygulanması

To determine the effect of the applications on macro-micronutrient content in leaves, N (%), P (%), K (%), Mg (%), Ca (%), Fe (ppm), Cu (ppm), Zn (ppm) and Mn (ppm) were measured. Leaf samples were first prepared for analysis after cleaning, drying, and grinding processes (Kacar, 1994). Except for the nitrogen element, other analyses were carried out on plant samples obtained by the wet combustion method. Nitrogen analysis was found as the percentage total N value in plant samples by Kjeldahl method. The phosphorus amount was measured by Spectrophotometer with vanodomolybdo phosphoric yellow color method (Lott et al., 1956), magnesium, calcium, and potassium amounts were measured by Atomic Absorption Spectrometer, and the results were given as percentage (Kacar, 1972). Manganese, copper, iron, and zinc amounts were also given as $mg.kg^{-1}$ (ppm) as a result of measurements made by Atomic Absorption Spectrometer (Lindsay & Norwell, 1978).

Statistical Analysis

In the study, phenological observations, fruit pomological characteristics, effects of applications on vegetative development, and macro-micronutrient element content in the leaf were investigated. The experiment was established using 6 applications, 3 replicates, and 3 trees in each replicate, totaling 54 trees. Irrigation was done by the drip irrigation method in all plots. Descriptive statistical methods, such as mean and standard deviation, were used to analyze each result. Results were expressed as mean ± standard deviation of three replicates, and differences between groups were distinguished at a significance level of $p \leq 0.05$. P values less than 0.05 were considered statistically significant at a 95% confidence interval. Statistical evaluation of the results obtained from each application was made according to variance analysis (ANOVA) and Duncan multiple comparison test in SPSS package program (version 20.0, SPSS Inc., Chicago).

RESULTS and DISCUSSION

Phenological observations were recorded from the bud swelling to leaf fall period.

Table 2. Phenological observations of Red Chief apple variety
Çizelge 2. Red Chief elma çeşidinin fenolojik gözlem sonuçları

Phenological Periods	2023
Bud swelling	08-10 April
Bud break	14-16 April
First bloom	20-24 April
Full bloom	25-29 April
End of bloom	29 April-1 May
Harvest date	5-10 October
Leaf fall date	11-25 November

In all applications, bud swelling started on April 8, and buds burst 6 days later. First flowering started 6 days after bud break and ended on May 1. Fruit harvest was made 164-169 days after full flowering, between October 5-10. Leaves started to turn yellow at the end of October, leaf fall started on November 11 and was completed on November 25. No effect of the applications on the phenological periods of the trees was observed.

Environmental conditions affect the phenological characteristics, fertilization biology, and pomological characteristics of apples. Therefore, in order to determine suitable cultivars for the region, the adaptation of different cultivars to the region should be investigated, and the cultivars should be selected according to the results obtained from this (Özbek, 1978). In his study in Tokat, Baytekin (2006) determined the bud break of the Red Chief variety on March 12, first flowering on April 21, full flowering on April 28, and end of flowering on May 3. In the study on the "Red Chief" apple variety in Niğde, bud swelling was determined as March 25, bud break as March 31, beginning of flowering as April 16, full flowering as April 22, end of flowering as April 28 and harvest date as September 20, and the period from full flowering to harvest was determined as 151 days (Ceylan, 2008). In his study conducted in Elmalı, Antalya, Arıkan (2020) stated that the bud swelling of the Red Chief apple cultivar occurred on March 17, the beginning of flowering on April 2, full bloom on April 7, and the end of flowering on April 17. In previous studies (Akgül & Başayiğit 2005; Ekinci 2010), it was determined that the Red Chief apple variety came to harvest in approximately 140-155 days. Kıraç (2016) determined the harvest date as October 14 in his study on the ' Red Chief'cultivar. It was reported that the harvest was made 163-169 days after full bloom in Kahramanmaraş conditions. Arıkan (2020) states that the harvest was made on September 27 in Elmalı, Antalya, 173 days after full bloom. Climatic conditions and altitude are effective on fruit ripening time.

Fruit pomological characteristics

For pomological analyses, measurements and analyses were carried out using 20 randomly selected fruits from each replication. The measurements of fruit weight, fruit width, length, and fruit skin color obtained from the applications in the study are given in Table 3. It was determined that the difference between the means of all these characteristics was statistically significant ($p<0.05$).

Table 3. Fruit weight, width, length, and fruit skin color values according to the different applications
Çizelge 3. Uygulamalardan elde edilen meyve ağırlığı, meyve eni, meyve boyu ve meyve kabuğu rengi değerleri

Applications	Fruit Weight (g)*	Fruit Width (mm)*	Fruit Leng (mm)*	Fruit Skin Color L*	a*	b*
1. Control	149.28±8.3 b	78.45±3.9 a	74.37±2.5 c	33.80±3.4 bc	22.13±3.9 b	7.53±2.2 b
2. 50% reduced	149.08±14.0 b	79.04±44.1a	74.94±3.9 bc	35.88±2.8 ac	27.28±4.3 a	9.37±1.6 a
3. 100% reduced	103.18±7.1 d	67.53±1.9 c	63.74±2.8 d	36.50±2.8 ab	22.65±3.1 b	9.31±1.5 a
4. Control + bacteria	170.57±16.2 a	80.06±3.0 a	79.36±3.2 a	32.92±3.4 c	23.03±3.2 b	7.41±2.1 b
5. 50% reduced+ bacteria	164.30±11.9 a	81.03±2.9 a	77.70±3.8 ab	35.47±3.2 ac	25.01±3.1 ab	9.26±1.2 a
6. 100% reduced+bacteria	135.69±9.6 c	74.49±2.0 b	74.95±3.9 bc	36.90±2.8 a	25.09±2.4 ab	9.97±1.5 a

*There is a statistical difference between the means shown with different letters in the same column ($p<0.05$).
* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır ($p<0,05$).

The highest fruit weight was obtained from Control (100% fertilizer dose) + Bacteria application (170.57 g) and 50% reduced fertilizer dose + Bacteria (164.30 g) applications. The lowest fruit weight was determined from 100% reduced fertilizer dose (without fertilizer) (103.18 g).

Karakurt (2006) determined the effects of Agrobacterium rubi A-18, Bacillus subtilis OSU-142, Burkholderia gladioli OSU-7, and Pseudomonas putida BA-8 bacterial strains on plant development, fruit set, fruit characteristics, and plant nutrient content of some 11-year-old apple cultivars grafted onto semi-dwarf (MM-106) rootstocks. It was determined that A-18 application caused an increase in fruit weight in Granny Smith and Starking Delicious cultivars, while other bacterial applications caused a decrease.

Baytekin & Akça (2011) found the average fruit weight of a 3-year-old Red Chief variety grafted on MM106 rootstock to be 235.80 g, Arıkan et al. (2015) found the average fruit weight of a 5-year-old Red Chief tree grafted on M26 rootstock to be 176.5 g, Öztürk et al. (2011) found the average fruit weight of a Red Chief variety grafted on M26 rootstock to be 167.42-180.54 g. Kıraç (2016) reported that fruit weights of the Red Chief apple variety varied between 84.8-176.5 g depending on the rootstocks, growing conditions, and age of the tree. Arıkan (2020) reported that the average fruit weights of the Red Chief apple variety were between 96.51-248.91 g (average 180.51 g) in their study. In the Yahyalı district of Kayseri province, Yaman et al. (2022) found the fruit weight of the Red Chief/M9 combination was 158.57 g, while the fruit weight of the Red Chief/MM106 was measured as 170.09 g. The effect of the bacterial application on fruit weight was positive, and this effect was found to be 6.18% in the M9 grafted and 14.51% in the MM106 grafted. The different sizes of the fruits vary according to genetics, cell number, intercellular space, cell size, climate, number of fruits on the tree, cultivation practices, and photosynthetic capacity of the trees.

In fruit growing, skin color is one of the important quality parameters. The ripening, health, decay, and harmful effects of the fruit can be understood by the color changes in the fruit skin. Color also affects the marketing of the products. Apple skin color is affected by many factors such as irrigation, nutrition, plant diseases, climate, and fruit set. In addition to these factors, day and night temperature differences are other factors affecting the fruit skin (Lakatos et al. 2012). In terms of L value, which determines fruit skin color brightness, the lowest was measured in Control (%100 fertilizer dose) +Bacteria (32.92) and Control (%100 fertilizer dose) (33.80) doses, while other applications were higher. The highest a value, which indicates a red color, was measured in 50% reduced fertilizer (27.28); 50% reduced fertilizer+bacteria (25.01) and 100% reduced fertilizer+bacteria (25.09) applications. The lowest b value, which indicates a yellow color, was obtained from Control (%100 fertilizer dose) (7.53) and Control (%100 fertilizer dose) +Bacteria applications (7.41). Fruit skincolors of Red Chief apple were measured as L=34.63, a=25.94 and b=10.30 by Küçüker (2010); L=34.70, a=27.26, b=8.73 by Ekinci (2010); L=39.99, a=26.13, b=16.79 by Baytekin & Akça (2011).

The measurements and analyses of the fruit firmness, total soluble solids (TSS), titratable acidity and yield per tree values obtained from the applications in the trial are given in Table 4. The difference between the means of these characteristics was found to be statistically significant (p<0.05).

Fruit firmness is one of the factors affecting the storage of fruits (Ekinci 2010; Öztürk et al. 2011; Kaynaş et al. 2011). Fruit firmness (kg/cm2) was determined in the highest bacteria-uncombined Control (100% fertilizer), 50% reduced fertilizer, and 100% reduced (without fertilizer) applications. The lowest fruit firmness was obtained from the Control (100% fertilizer) + bacteria application with 6.50 kg/cm2. The average fruit firmness of Red Chief apple was determined as 11.62 lb (5.27 kg/cm2) by Arıkan et al. (2015), 8.82 lb (4.00 kg/cm2) by Satıcı (2011), and between 15.58 and 17.94 lb by Öztürk et al. (2011). Arıkan (2020) measured the average fruit firmness as 7.18 kg/cm2 (15.82 lb). The values obtained were found to be above the values found by Arıkan et al. (2015) and Satıcı (2011), and similar to the values of Öztürk et al. (2011) and Arıkan (2020). Different results show that fruit firmness may be caused by many factors such as climate, altitude, rootstock, and harvest time.

Total soluble solids (TSS), an important indicator of ripening in fruits, play an important role in the aroma of fruits due to the sugars in the fruit as well as ripening (Kaynaş et al. 2011). TSS was measured at the highest in the Control (100% fertilizer) + bacteria applications Brix value of 20.30%, and in the 50% reduced + bacteria applications, with a Brix value of 19.90%. In the studies, the amount of TSS was determined as 14.16% by Satıcı (2011), 14.73% by Kıraç (2016), 13.55% by Baytekin & Akça (2011), 10.3% by Öztürk et al. (2011), 11.84% by Arıkan et al. (2015), and 12.43% by Arıkan (2020). Yaman et al. (2022) measured the TSS value as 13.07% in fruits grafted onto M9 rootstock of the Red Chief variety and 13.77% in fruits grafted onto MM106 rootstock. While the effect of bacteria application on TSS was negative, this effect was found to be -7.01% in fruits grafted onto M9 and -7.50% in fruits grafted onto MM106. The ripening process of the fruit begins with the picking of the fruit, and the TSS ratio gradually decreases as time passes (Satıcı, 2011). Wang et al. (2022) found that soluble sugar was higher in low fertilizer and DMPP added treatments. The results revealed that 40% N reduction combined with DMPP (TN60% + D) improved fruit quality on the basis of the traditional N application rate (P < 0.05).

Table 4. Fruit firmness, TSS, TA, and yield values obtained from the applications

Çizelge 4. Uygulamalardan elde edilen meyve eti sertliği, suda çözünebilir kuru madde, titrasyon asitliği ve verim değerleri

Applications	Fruit firmness ($kg.cm^{-2}$)*	Total soluble solids (TSS) (%)*	Titratable acidity (TA) (%)*	Yield ($kg.tree^{-1}$)*
1. Control	8.17±0.8 a	18.88±0.2 bc	0.59±0.02 a	19.63±1.1 ab
2. 50% reduced	7.89±0.2 a	17.47±0.9 c	0.47±0.03 b	16.78±1.4 bd
3. 100% reduced	7.61±0.2 ab	18.30±0.7 c	0.37±0.02 c	14.78±0.7 d
4. Control + bacteria	6.50±0.5 c	20.30±1.0 a	0.40±0.01 c	22.35±1.0 a
5. 50% reduced + bacteria	6.77±0.5 bc	19.90±0.2 ab	0.39±0.02 c	18.30±2.9 bc
6. 100% reduced + bacteria	6.83±0.6 bc	18.03±1.0 c	0.37±0.01 c	15.92±1.3 cd

*There is a statistical difference between the means shown with different letters in the same column (p<0.05).

* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır (p<0,05).

The highest titratable acid amount was determined as 0.59% in the Control (100% fertilizer) application. In similar studies, the titratable acidity was determined as 0.23% by Satıcı (2011), 0.39% by Sabır et al. (2011) and Baytekin & Akça (2011), 0.33% by Arıkan et al. (2015), and 0.67% by Arıkan (2020). Yaman et al. (2022) found the titratable acidity value as 1.00% and -5.67% negative bacterial effect in the seedling grafted onto Red Chief/M9 rootstock, while they found 0.80% and 1.38% positive bacterial effect in the seedling grafted onto Red Chief/MM106 rootstock. In studies conducted with different fruit species, it was determined that bacterial application significantly reduced the TEA and SÇKM contents in apple (Karakurt & Aslantaş, 2010), strawberry (Eşitken et al., 2010), quince (Arıkan et al., 2013), and pomegranate (Acar, 2018) species.

The highest yield values per tree obtained by collecting the fruits from the trees of each replication separately were obtained from the Control (%100 fertilizer) +bacteria (22.35 kg/tree) and Control (%100 fertilizer) applications (19.63 kg/tree). The lowest yield value was found in the 100% reduced fertilizer (without fertilizer) application. Kıraç (2016) found the yield per tree of Red Chief apple on MM106 rootstock to be 20-25 kg, Einhorn & Caspari (2004) found the yield per tree to be 20.9-27.7 kg on M26 rootstock and Lombardini et al. (2004) found the yield per tree to be 16.17 to 20.8 kg on M9 rootstock. Arıkan (2020) obtained a yield value of 99.34 kg/tree from Red Chief apple on MM111 rootstock. In the study of Wang et al. (2022), TN40% yield was found to be 12.53% lower than TN. The results showed that reducing the N application rate to 200 kg ha−1 (TN40%) negatively affected the yield and economic benefit. While the yield and yield value decreased with the reduction of N rate in the DMPP addition application, the agronomic benefit first increased and then decreased. The reason for the different yield amounts may be due to planting density, rootstock differences, and growing conditions.

Effect of applications on vegetative development

In order to determine the effect of applications on the vegetative development of trees, leaf and shoot characteristics were taken. The difference between the means of all characteristics except original leaf weight, leaf chlorophyll amount, and shoot diameter was found to be statistically significant ($p<0.05$) (Table 5).

Table 5. Leaf area, leaf fresh and dry weight, specific leaf weight, leaf anthocyanin amount, and leaf chlorophyll amount values were obtained from the applications

Çizelge 5. Uygulamalardan elde edilen yaprak alanı, yaprak yaş ve kuru ağırlığı, özgül yaprak ağırlığı, yaprak antosiyanin miktarı ve yaprak klorofil miktarı

Applications	Leaf Area (cm^2) *	Leaf Fresh Weight (g) *	Leaf Dry Weight (g) *	Specific leaf weight ($g.cm^{-2}$) NS	Leaf Anthocyanin (ACI) *	Leaf Chlorophyll (SPAD) NS
1. Control	55.88±5.5 a	1.29±0.2 a	0.577±0.1 a	0.0103±0.001	11.55±3.2 ab	50.86±4.6
2. 50% reduced	53.19±7.1 ab	1.27±0.2 a	0.574±0.1 a	0.0108±0.002	11.42±2.4 ab	52.35±5.0
3. 100% reduced	46.79±7.3 c	1.10±0.2 b	0.472±0.1 c	0.0101±0.002	10.47±1.8 b	49.85±4.4
4. Control + bacteria	51.97±7.9 ac	1.19±0.2 ab	0.536±0.1 ab	0.0103±0.003	12.78±2.2 a	50.99±4.8
5. 50% reduced + bacteria	49.23±7.3 bc	1.06±0.2 b	0.495±0.1 b	0.0101±0.002	11.90±2.6 ab	50.26±3.1
6. 100% reduced + bacteria	49.59±4.7 bc	1.08±0.1 b	0.499±0.1 b	0.0101±0.002	12.08±1.3 ab	50.85±2.7

*There is a statistical difference between the means shown with different letters in the same column ($p<0.05$). NS: Not significant

* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır (p<0,05).

NS: Önemli değil

The lowest leaf area was obtained in the 100% reduced (without fertilizer) application. Yaman et al. (2022) found the leaf area value as 30.79 cm^2 and 2.98% positive bacterial effect in the Red Chief/M9 combination, while it was 27.92 cm^2 and 8.67% positive bacterial effect in the Red Chief/MM106 combination.

Leaf fresh and dry weight were measured as the highest in Control (100% fertilizer), 50% reduced fertilizer and Control (100% fertilizer) + bacteria applications. Although there was no statistically significant difference between the applications in terms of specific leaf weight calculated by dividing leaf dry weight by leaf area (Zokaee-Khosroshahı et al., 2014), the highest value was obtained from 50% reduced fertilizer application. The lowest leaf anthocyanin amount was determined in 100% reduced (without fertilizer) application. All other applications were statistically in the same group. Yaman et al. (2022) found the SPAD value to be 56.35 and -0.58% negative bacterial effect in the Red Chief/M9 combination and 54.47 and -2.45% negative bacterial effect in the Red Chief/MM106 combination.

Effect of applications on macro-micronutrient content in leaves

When the effects of applications on macro and micronutrient elements in leaves were examined, the differences between the means were found to be statistically significant ($p<0.05$) (Table 6-7).

Leaf nitrogen (N) content was highest in Control (100% fertilizer), Control (100% fertilizer) + bacteria, and 50% reduced + bacteria applications. The highest P content was obtained from Control (100% fertilizer) and Control (100% fertilizer) + bacteria applications. The highest K content was measured in 50% reduced fertilizer application. Ca content was higher in Control (100% fertilizer) + bacteria, combined with bacteria; 50% reduced fertilizer + bacteria, and 100% reduced (without fertilizer) + bacteria applications. The lowest Mg content was found in 100% reduced (without fertilizer) and 100% reduced (without fertilizer) + bacteria applications.

The lowest amount of Fe was measured in the 100% reduced (without fertilizer) application, while the other applications were statistically in the same group. The highest amount of Cu was obtained from the Control (100% fertilizer) application and the 50% reduced fertilizer application. The highest amount of Zn was determined in the 50% reduced fertilizer application. The lowest amount of Mn was found in the 50% reduced fertilizer and 100% reduced (without fertilizer) applications (Table 7).

Table 6. N, P, K, Ca and Mg values in the leaves obtained as a result of the applications
Çizelge 6. Uygulamalar sonucunda elde edilen yapraklardaki N, P, K, Ca ve Mg değerleri

Applications	N (%)*	P (%)*	K (%)*	Ca (%)*	Mg (%)*
1. Control	1.619±0.002ab	0.203±0.02 a	0.235±0.005 c	2.651±0.08 d	0.059±0.0005 a
2. 50% reduced	1.597±0.04 b	0.132±0.01 b	0.290±0.01 a	3.265±0.16 c	0.059±0.008 a
3. 100% reduced	1.532±0.02 c	0.121±0.01 b	0.220±0.01 c	3.298±0.11 bc	0.048±0.005 b
4. Control + bacteria	1.618±0.03 ab	0.191±0.02 a	0.270±0.01 b	3.467±0.03 ab	0.053±0.002 ab
5. 50% reduced + bacteria	1.655±0.02 a	0.140±0.003 b	0.235±0.02 c	3.459±0.003 ab	0.057±0.002 a
6. 100% reduced + bacteria	1.591±0.03 b	0.138±0.01 b	0.220±0.01 c	3.518±0.14 a	0.045±0.007 b

There is a statistical difference between the means shown with different letters in the same column (p<0.05).
* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır (p<0,05).

Table 7. Fe, Cu, Zn and Mn values in the leaves obtained as a result of the applications
Çizelge 7. Uygulamalar sonucunda elde edilen yapraklardaki Fe, Cu, Zn ve Mn değerleri

Applications	Fe (ppm) *	Cu (ppm) *	Zn (ppm) *	Mn (ppm) *
1. Control	202.0±6.0 ab	22.40±2.2 a	16.90±1.3 b	168.0±5.2 a
2. 50% reduced	186.0±24.0ab	20.40±2.2 a	20.00±1.4 a	137.0±4.5 b
3. 100% reduced	181.0±19.0 b	9.90±0.9c	14.10±1.1 b	133.0±5.0 b
4. Control + bacteria	198.0±8.0 ab	14.50±1.3 b	17.00±2.4 b	168.0± 5.3 a
5. 50% reduced + bacteria	215.0±19.0 a	13.15±0.75 b	15.07±2.1 b	170.0±4.6 a
6. 100% reduced + bacteria	199.0±11.0 ab	12.60±0.2 b	15.00±0.8 b	154.0±4.0 ab

* There is a statistical difference between the means shown with different letters in the same column (p<0.05).
* Aynı sütunda farklı harflerle gösterilen ortalamalar arasında istatistiksel olarak fark vardır (p<0,05).

Karakurt (2006) determined the lowest (1.57%) value in leaf nitrogen content in the Granny Smith variety, and the highest (2.12%) value in Starkrimson Delicious variety. It was found that Starking Delicious (1724 ppm) and Granny Smith (1630 ppm) had the lowest value, while Golden Delicious (3895 ppm) had the highest value in phosphorus content. It was reported that OSU-142, OSU-7, and BA-8 applications decreased the phosphorus content in Starking Delicious variety, while A-18 caused an increase. It was determined that Starking Delicious (19359 ppm) and Granny Smith (18482 ppm) had the lowest value, while Starkspur Golden Delicious had the highest value (31790 ppm). In terms of magnesium content, Golden Delicious variety had the lowest (1186 ppm), Starkspur Golden Delicious variety had the highest (1327 ppm), and OSU-142 bacteria application (1282 ppm) had higher magnesium content than the control and other bacteria applications. He found the variety x application interaction on calcium content to be very important. It was determined that sodium content varied between 566-644 ppm, depending on variety and bacterial applications. It was determined that iron content was the lowest in Starking Delicious (17.7 ppm) and the highest in Golden Delicious (45.6 ppm) and OSU-142 (32.7 ppm) application increased the iron content compared to the control (27.9 ppm) and other bacterial applications. It was determined that Starking Delicious (25.5 ppm) and Granny Smith (26.3 ppm) had the lowest manganese content, and Starkspur Golden Delicious (54.7 ppm) had the highest manganese content. Golden Delicious (5.9 ppm) had the lowest zinc content, and Starkspur Golden Delicious (17.2 ppm) had the highest content, while A-18 (17.2 ppm) application among the bacterial applications provided a greater increase in leaf zinc content compared to the control (12.9 ppm) and other bacterial applications. Copper content was found to be lowest in Granny Smith (3.2 ppm) and highest in Golden Delicious (6.8 ppm).

Karlıdağ et al. (2007) determined that bacterial applications had a positive effect on all nutrient element contents (N, P, K, Ca, Fe, Mn, and Zn) except Mg in the leaf in their studies, where they applied three different bacterial strains and combinations to the Granny Smith apple variety. Kotan et al. (2021) examined the contents of some nutrients in the leaves with the microbial fertilizer they used in apple sapling cultivation. They reported that the use of bacteria caused an increase in all parameters in the leaf except Cu (%-19.91) compared to the control, the lowest of these increases was in the amount of Zn in the leaf with 0.33%, while the highest increase occurred in the amount of Mg with 69.69%, followed by P (%36.54), N (%27.74), K (%23.64), Mn (%20.52), Ca (%15.17) and Fe (%6.99).

The results of the statistical analysis of some fruit and leaf characteristics are given in Table 8, and the results of the statistical analysis of leaf macro and micronutrients are given in Table 9.

Table 8. Statistical results of applications on some features (Degree of freedom, F value, p value and η^2 value)

Çizelge 8. Uygulamaların bazı özellikler üzerine istatistiksel sonuçları (Serbestlik derecesi, F değeri, p değeri ve eta² değeri)

Features	Degree of freedom	F value	Sig.(P value)	η^2
Fruit Weight (g)	59	42.714	0.000	0.798
Fruit Width (mm)	59	26.535	0.000	0.710
Fruit Length (mm)	59	25.663	0.000	0.704
Fruit Skin Color L*	59	2.608	0.035	0.195
Fruit Skin Color a*	59	3.267	0.012	0.232
Fruit Skin Color b*	59	3.803	0.005	0.260
Yield (kg.tree^{-1})	17	9.054	0.001	0.790
Fruit firmness (kg.cm^{-2})	17	5.793	0.006	0.707
Total soluble solids (TSS)(%)	17	6.235	0.004	0.722
Titratable acidity (%)	17	57.155	0.000	0.959
Leaf Fresh Weight (g)	89	4.015	0.003	0.193
Leaf Dry Weight (g)	89	3.665	0.005	0.179
Leaf Area (cm^2)	89	3.322	0.009	0.165
Leaf Anthocyanin (ACI)	89	1.624	0.162	0.088
Leaf Chlorophyll (SPAD)	89	0.621	0.684	0.036
Specific leaf weight (g.cm^{-2})	89	0.446	0.815	0.026

Table 9. Statistical results of the applications on macro-micronutrients in leaves (Degree of freedom, F value, p value, and η^2 value)

Çizelge 9. Uygulamaların yapraklardaki makro-mikro besin elementleri üzerine istatistiksel sonuçları (Serbestlik derecesi, F değeri, p değeri ve eta² değeri)

Macro-micro nutrients	**Degree of freedom**	**F value**	**Sig.(P value)**	**η^2**
N (%)	17	7.857	0.002	0.766
P (%)	17	22.077	0.000	0.902
K (%)	17	22.708	0.000	0.904
Ca (%)	17	28.726	0.000	0.923
Mg (%)	17	4.432	0.016	0.649
Fe (ppm)	17	1.732	0.202	0.419
Cu (ppm)	17	33.208	0.000	0.933
Zn (ppm)	17	5.223	0.009	0.685
Mn (ppm)	17	3.564	0.033	0.598

CONCLUSION

In plant production, differences in ecological conditions cause differences in phenological, pomological, and vegetative growth values of fruits. In general, fruit characteristics are affected by the total temperature of the region where the plant is grown, altitude, direction, vegetation period duration, cultivation techniques, and the time from full bloom to harvest. Fruits grown in high-altitude areas and plateaus are more durable than those grown in low-altitude areas and bottom lands, and apples grown in hot regions are larger and more flattened than those grown in cool regions. Fruits are smaller and harder as a result of the cells not being able to develop in arid regions due to a lack of water. Ecological conditions must be suitable for the genetic characteristics of the plant to emerge (Karaçalı, 1993; Westwood, 1993).

Bacteria have the ability to increase the synthesis of hormones such as auxin, gibberellin, and cytokinin in the plant, thus increasing the vegetative growth and development of the plant (Aslantaş et al., 2007; Eşitken et al., 2010; Gerçekcioğlu et al., 2018). As a result of bacterial application, the increase in the plant's leaf area increases photosynthetic efficiency, and fruit quality criteria are positively affected in parallel. Selvi et al. (2020) stated that the use of bacteria, which has productivity and low cost, is in line with the universal principles of sustainable agriculture. As a result, it was concluded that microbial fertilizers containing plant growth-promoting bacteria can be used in apples. Replication of the study in different climatic and geographical conditions may increase the extensibility of the results. It is recommended to disseminate the study to adopt microbial fertilizers as a sustainable alternative in agriculture.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the article declare that there is no conflict of interest between them.

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