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Effects of Rhizobacteria Application on Leaf and Fruit Nutrient Content of Different Apple Scion–Rootstock Combinations

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Abstract: The plant pomological characteristics and physiological behaviors of genotypes in modern apple cultivation could be different depending on the use of rootstock, changing growth ecology and application of biological control agents. The aim of this research was to determine the effects of rhizobacteria application on leaf and fruit nutrient contents in different apple scion–rootstock combinations. This study was carried out with seven standard cultivars (Scarlet Spur, Red Chief, Fuji, Jeromine, Galaxy Gala, Granny Smith, and Golden Reinders) budded on M.9 and MM.106 rootstocks. In the experiment, trees were sprayed by a nitrogen + phosphorus solvent rhizobacteria three times, with an interval of 15 days in the spring period. The effect of rhizobacteria application on leaf and fruit nutrient contents was statistically significant and provided generally significant positive contributions, except for leaf Mg content. Comparing both rootstocks, the positive effect of bacterial application was higher on the M.9 rootstock for leaf N and B content and fruit N and Fe content, and on the MM.106 rootstock for other nutrient content. While the effects of bacterial application on the basis of cultivars were generally positive, the highest positive contribution was made in leaf P content (10.7%) and fruit Mn content (32.1%) of the Fuji cultivar. Considering the total increase in nutrients in scion–rootstocks combination, rhizobacteria application had a positive effect on the leaf nutrient contents in Golden Reinders/MM.106, but not leaf K content. The highest increases in leaves of scion-rootstock combinations were determined as 4.0% in N content in Granny Smith/M.9, 14.1% in P content in Scarlet Spur/MM.106, 7.1% in K content in Fuji/MM.106, 4.4% in Ca content in Jeromine/M.9, and 14.0% in Mg content in Granny Smith/MM.106. The highest increase in fruit nutrient contents was between 4.9% (N content) and 13.5% (Ca content) for macro elements, and between 9.5% (Cu content) and 41.8% (Mn content) for microelements. The results of the present study may provide significant leads for further studies on this subject.

Keywords apple cultivars; rootstock; rhizobacteria application; plant nutrient contents

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1. Introduction

The apple is one of the most important and popular fruit species in the world, both in terms of trade and production. In apple cultivation, it is possible to obtain a high yield and quality products, especially with a correct and balanced fertilization, together with growing techniques such as pruning, irrigation, control of diseases and pests, etc. As a result of long-term cultivation of Turkey agriculture soils and an insufficient application of additives that could improve the soil structure, the productivity of the soil has decreased. On the other hand, the agriculture soils have become unproductive due to the excessive and unconscious use of some chemical fertilizers and the effect of natural conditions [1].

The use of beneficial microorganisms instead of synthetic chemicals in agriculture can support plant growth, prevent damage to the environment, and ameliorate soil fertility [2]. Plant-growth-promoting microorganisms are generally grouped as biofertilizers that increase the nutrient ratio in the plant, phyto stimulants that promote plant growth with plant hormone production, rhizoremediators that breakdown recalcitrant toxic pollutants, and biopesticides that control diseases by producing antibiotics and antifungal metabolites. The application of biofertilizers and biocontrol agents in agriculture has increased, especially in recent years [3].

Plant-growth-promoting bacteria (PGPR) are free-living organisms in the soil, and they are very useful in crop production. These rhizobacteria are generally included in the species *Pseudomonas* spp., *Azospirillum* spp., *Burkholderia* spp., *Bacillus* spp., *Enterobacter* spp., *Rhizobium* spp., *Erwinia* spp., *Serratia* spp., *Alcaligenes* spp., *Arthrobacter* spp., *Acinetobacter* spp., and *Flavobacterium* spp. [4–6]. These organisms have many beneficial effects on plant growth and productivity. They promote plant growth by enhancing nutrient accumulation to the plants [7,8]. In recent years, the use of rhizobacteria in sustainable agricultural has increased to improve soil fertility and crop productivity and to reduce the negative effects of chemical fertilizers on the environment. Rhizobacteria increase the resistance of the plant against biotic and abiotic stress conditions such as weeds [9], drought stress [10], heavy metals [11], and salt stress [12,13], which adversely affect plant growth. It is reported that rhizobacteria provide an increase in yield and contribute to many morphological and physiological characteristics such as seed germination [14], root and shoot growth [15], leaf area, and chlorophyll, protein, N, and Mg contents in plants [16,17]. An increase in the amount of antioxidant enzymes and some hormones is observed in plants under different stress conditions. Catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) enzyme amounts, as well as salicylic acid and gibberellic acid hormone amounts, increase while abscisic acid amount decreases [18].

Rootstock is extremely important in fruit growing, and it is an important parameter that positively or negatively influences the plant's resistance to biotic and abiotic stress factors, fruit yield and quality, and plant characteristics, such as the growth power of the tree. Factors such as plant age, development status, plant type, variety, and root system structure can affect the amount of nutrients that plants have removed from the soil to varying degrees. The rootstock and cultivar characteristics also have a significant effect on the nutrient content of the plants [19]. The most widely used and commercially produced rootstocks in the world are M.9 and MM.106 rootstocks [20]. In trials with clone rootstocks in Turkey, the highest yield was obtained from M.9 and MM.106 rootstocks, and these rootstocks were recommended for Turkey as well [21,22]. Since the M.9 rootstock is surface rooted, it definitely needs support systems. The MM.106 rootstock is a semi-dwarfing rootstock with high productivity and good adhesion to the soil.

All morphological, physiological, and biochemical processes occurring in plants are affected by the individual, or combination of, rootstock and scion. Leaf mineral nutrient composition of cultivars generally differs as a function of rootstock–scion combination and ecological conditions. Thus, many researchers conducted experiments towards understanding the changes in nutrient uptake of different cultivars at different physiological stages under changing environmental conditions [23–25].

The objective of this study was to investigate the rhizobacteria application effects on leaf and fruit nutrient element composition in different apple scion–rootstock combinations.

2. Material and Methods

2.1. General Conditions of Place, Climate, and Year of Investigations

The study was carried out in 2020 and 2021, in the Develi Plain of Kayseri. The Develi Plain has an area of approximately 1000 km^2. It was formed by the volcanic movements of Mount Erciyes. The climate in the region is continental—generally cold and snowy in winters and hot and dry in summers. The daily air temperature and humidity values in the region at the application times are as follows: temperature: 15.8 °C, 17.7 °C, and 19.3 °C, respectively; humidity: 58%, 54% and 48%, respectively. The general characteristics of the orchard are as follows: the orchard was planted in 2014 at 0.75 m within a row and 4.0 m between a row with M.9 rootstock, and at 1.5 m within a row and 4.0 m between a row with MM.106 rootstock. The fertilizer application was carried out together with drip irrigation (fertigation system) to this area every year. The application of the fertilization program in the orchard is as follows. Before flowering: Urea (1 kg/ha), Monoammonium Phosphate (MAP, 0.3 kg/ha, 3 times), Magnesium sulfate (1 kg/ha), and chelated iron (0.5 kg/ha), in the first flowering time (in May); foliar fertilizer (MC EXTRA, 0.2 lt/ha), Urea (0.5 kg/ha), MAP (0.2 kg/ha), Magnesium sulfate (0.5 kg/ha), and chelated iron (0.5 kg/ha), in June; Potassium sulfate (0.2 kg/ha), Magnesium sulfate (0.5 kg/ha), and foliar fertilizers (P-Zn-SMART; 0.1 lt/ha, CALTRAK-CaO_2; 0.3 lt/ha), in July; Potassium sulfate (0.2 kg/ha) and foliar fertilizers (MANNI PLEX-Mn; 0.1 lt/ha, P-Zn-SMART; 0.1 lt/ha, CALTRAK-CaO_2; 0.3 lt/ha), in August; Potassium sulfate (0.2 kg/ha) and foliar fertilizer (CALTRAK-CaO_2; 0.5 lt/ha), after harvest; foliar fertilizer (P-Zn-SMART; 0.1 lt/ha).

2.2. Planting Material

Experiments were carried out on with standard apple cultivars (Scarlet Spur, Red Chief, Fuji, Jeromine, Galaxy Gala, Granny Smith, and Golden Reinders) budded on 2 rootstocks (M.9 and MM.106). The trees were budded at the height of approximately 15–20 cm.

2.3. Soil Analysis

Soil samples were taken as 3 samples from a 0–30 cm and a 30–60 cm depth and analyzed before applications in the spring of 2020. The minimum and maximum results of nutrient content and physical and chemical properties of soil are given in Table 1. According to the results of the analysis, it has been determined that the orchard soil has a loamy texture, and there is no salt problem that will limit plant production. The pH of the soil is in the mild alkaline class, and it is in the middle class in terms of lime and organic matter content. All nutrient contents were found to be sufficient.

Table 1. Nutrient content and physical and chemical properties of apple orchard soil.

Soil Depth	P	K	Ca	Mg	Mn	Zn	Fe	Cu
				mg/kg				
0–30 cm	11.5–15.1	151.3–241.5	1479.3–1750.0	228.1–258.9	18.2–29.7	2.66–3.58	1.10–1.53	1.38–2.12
30–60 cm	11.5–13.2	132.8–194.2	1512.0–1815.9	211.0–231.8	17.5–22.2	2.52–3.15	0.97–1.50	1.31–1.98
	Texture class	EC (dS/m)	pH	Lime (%)	Organic matter (%)		Bacteria density (cfu/mL)	
0–30 cm	Loamy	0.39–0.43	8.2–8.3	6.88–7.13	2.15–2.38		0.309×10^6–0.330×10^6	
30–60 cm		0.29–0.31	8.1–8.2	7.09–7.27	2.11–2.15		-	

2.4. Preparation of Bacteria Solutions

Azospirillum sp-245 and *Bacillus megaterium* M3 were used as rhizobacteria. Bacteria were grown on Nutrient Agar and transferred to 250 mL flasks containing Nutrient Broth with 15% glycerol. They had grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Darmstadt, Germany). The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 CFU/mL.

2.5. Treatment

Bacterial treatment with *Azospirillum* sp-245 + *Bacillus megaterium* M3 was performed with the bacterial suspensions of 10^8 CFU/mL to the canopy projectional areas of the trees 3 times, with an interval of 15 days after full flowering. Control plants were sprayed with sterile water. The experiment was established with 3 replications for each scion–rootstock combination, and there were 5 trees in each replication. In the experiment, bacteria were applied to a total of 210 plants.

2.6. Leaf and Fruit Mineral Analysis

The effects of bacterial treatments were evaluated by determining nutrients content in the fruits and leaves. The method applied by Coskun and Askin [26] was used to determine the nutrients content of fruit samples. Fruit sampling was carried out from a height of approximately 1.5 from the soil in the control and application trees, with 10 fruits in each replication at full harvest. The stage in which starch is degraded by 50% in the fruit was accepted as the ripening period [27]. The leaf sampling was completed from the middle parts of the non-fruiting shoots with 15–20 leaves in each replication, at the end of July [28]. Leaf and fruit samples were washed with water to remove the contaminants and distilled water as a preliminary step. The fruit samples were cut into small pieces, and the leaf and fruit samples were then dried at 65–70 °C until the weight stabilized. The samples were ground to be less than 0.5 mm in size. The total nitrogen content of fruit and leaf samples burned by the Kjeldahl method was determined by steam distillation [29]. To determine the amount of other nutrients, the samples were thawed by the dry combustion method, and these nutrients concentration of the samples were read in the ICP-OES instrument [30].

2.7. Data Analysis

All data in the present study were subjected by analysis of variance (ANOVA) and means were separated by Tukey's multiple range tests. There were no statistical differences between years; therefore, the data were pooled.

3. Results

3.1. Leaf Nutrient Contents

The results of leaf nutrient content in apple cultivars on different rootstocks in the control group are shown in Table 2. The effect of rootstock, cultivar, and scion–rootstock combination on the nutrient content of leaves differs statistically. The effects of cultivar and scion–rootstock combination on leaf nutrients emerged more clearly than rootstock. The rootstock effect was found to be insignificant except for the K, Zn, Cu, and B contents; the K and B contents were higher on the MM.106 rootstock and the Zn and Cu contents on the M.9 rootstock. The effects of the cultivar on the leaf nutrient contents were statistically insignificant in K and Ca elements, but significant in other elements. It was determined that the highest values on leaves were found in Jeromine for N, Fe, Mn, and Zn, Red Chief for P and Cu, and Scarlet Spur for Mg and B. The leaf nutrient contents differed statistically according to scion–rootstock combinations, but not nitrogen. The leaf N level varied between 1.93–2.32% for Granny Smith/M.9 and Jeromine/MM.106. The highest leaf nutrient contents were determined in the combinations of Red Chief/M.9 for the P element (0.18%), Golden Delicious/MM.106 for K (1.85%) and Ca elements (1.53%), and Scarlet

Spur/M.9 and Galaxy Gala/MM.106 for the Mg element (0.37% and 0.36%, respectively). Considering the micronutrient content obtained, these values were varied between 68.81 mg/kg (Golden Reinders/MM.106) and 122.42 mg/kg (Jeromine/M.9), 38.93 mg/kg (Galaxy Gala/M.9) and 96.69 mg/kg (Jeromine/MM.106), and 24.92 mg/kg (Fuji/MM.106) and 62.83 mg/kg (Jeromine/M.9) for Fe, Mn, and Zn elements, respectively. The highest Cu and B contents were determined in Galaxy Gala/M.9 (13.20 mg/kg) and Scarlet Spur/MM.106 (56.10 mg/kg) combinations, respectively, and the lowest in Galaxy Gala/MM.106 (5.38 mg/kg) and Granny Smith/M.9 (29.42 mg/kg) combinations, respectively.

Table 2. Effect of rootstocks, cultivars, and scion–rootstock combinations on leaf nutrients in the control application.

	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu	B
	% (dw)					mg/kg (dw)				
Rootstock										
M.9	2.11	0.14	1.60b	0.29	1.34	94.03	62.97	46.17a	8.79a	37.05b
MM.106	2.09	0.13	1.69a	0.32	1.38	89.47	60.40	39.27b	7.02b	47.16a
Cultivar										
Scarlet Spur	2.03bc	0.14bc	1.65	0.36a	1.28	84.94c	77.91b	50.05b	7.03c	52.19a
Fuji	2.21ab	0.13bc	1.71	0.27b	1.44	90.50bc	49.95de	28.18e	6.33c	40.43c
Granny Smith	2.03bc	0.14bc	1.58	0.27b	1.30	96.77b	51.03de	33.37de	6.49c	34.76d
Galaxy Gala	2.12abc	0.12cd	1.63	0.32ab	1.42	82.63c	47.55e	47.86bc	9.29ab	33.91d
Golden Reinders	2.04bc	0.11d	1.75	0.31ab	1.40	85.24c	60.11c	44.14c	7.05c	37.89cd
Red Chief	1.97c	0.17a	1.56	0.29b	1.41	95.32b	54.60cd	34.13d	10.17a	46.54b
Jeromine	2.29a	0.16ab	1.65	0.31ab	1.27	106.85a	90.63a	61.30a	9.00b	49.02ab
Scion–rootstock combination										
Scarlet S./M.9	2.08	0.16ab	1.55bcd	0.37a	1.46abc	82.65defg	87.52a	52.88abc	7.21ef	48.28bc
Fuji/M.9	2.27	0.15abc	1.77ab	0.29ab	1.42abcd	80.40efg	44.13ef	31.45fgh	6.73efg	34.82ef
G.Smith/M.9	1.93	0.13bcde	1.59bcd	0.29ab	1.35bcde	88.88cdef	56.18cd	34.03fgh	6.49efg	29.42g
G.Gala/M.9	2.24	0.11cde	1.50d	0.28ab	1.36abcde	87.92cdef	38.93f	50.52bc	13.20a	29.59fg
Golden R./M.9	1.99	0.10e	1.65abcd	0.28ab	1.27def	101.66bc	71.37b	51.86bc	7.91de	31.81fg
Red Chief/M.9	1.98	0.18a	1.53cd	0.26b	1.32cde	94.31bcde	58.07bc	39.61def	10.88b	42.57cd
Jeromine/M.9	2.27	0.15abc	1.64abcd	0.29ab	1.20ef	122.42a	84.57a	62.83a	9.15cd	42.86cd
Scarlet/MM.106	1.98	0.13bcde	1.74abc	0.35ab	1.11f	87.22cdef	68.30b	47.22cd	6.86efg	56.10a
Fuji/MM.106	2.15	0.12bcde	1.64abcd	0.26b	1.47abc	100.61bc	55.78cde	24.92h	5.94fg	46.04c
G.Smith/MM.106	2.13	0.14abcd	1.57bcd	0.25b	1.26def	104.66b	45.89def	32.70fgh	6.48efg	40.10de
G.Gala/MM.106	2.01	0.13bcde	1.75abc	0.36a	1.47abc	77.34fg	56.16cd	45.21cde	5.38g	38.23e
Golden/MM.106	2.09	0.11cde	1.85a	0.33ab	1.53a	68.81g	48.86cdef	36.42efg	6.19fg	43.97cd
R.Chief/MM.106	1.96	0.16ab	1.60bcd	0.32ab	1.50ab	96.34bcd	51.13cde	28.65gh	9.46bc	50.51b
Jeromine/MM.106	2.32	0.16ab	1.67abcd	0.34ab	1.33cde	91.29bcdef	96.69a	59.78ab	8.85cd	55.19a

The difference between the averages indicated by different letters in the same column, separately, for rootstock, cultivar, and scion–rootstock combination is significant ($p < 0.05$). The absence of letters indicates no statistical significance.

The contribution of rhizobacteria application on the leaf nutrient contents was statistically significant in rootstocks (except for Mg, Ca, Mn, and Zn elements), cultivars (except

for B element) and scion–rootstock combinations (Table 3). In terms of rootstocks, the positive contribution of bacterial application was more obvious for the M.9 rootstock for N and B content, and for the MM.106 rootstock for P, K, Fe, and Cu content. The effects of bacterial application on leaf nutrient content differed according to the cultivars, and the effect was generally positive. Bacteria application made the highest positive contribution with 10.7% in P content of Fuji cultivar. The Fuji cultivar was followed by the Mg content of the Granny Smith cultivar with an 8.6% positive contribution. On the other hand, bacteria application provided the most positive contribution by increasing three nutrients in the leaves of Gala (for Fe, Mn, and Cu contents) and Jeromine (for Mg, Fe, and Mn contents) cultivars by over 5%. These cultivars were followed by Scarlet Spur (for P and Fe contents) and Golden Reinders (for P and Zn contents) cultivars with the increase in two nutrient elements. While rhizobacteria had different effects on leaf nutrient contents according to scion–rootstock combinations, it was generally positive effect except for the Mg content. On the other hand, the rhizobacteria had no effect on macronutrient uptake in some scion–rootstock combinations, for example, in combinations of Fuji/M.9, Jeromine/M.9, Scarlet Spur/MM.106, and Jeromine/MM.106 for N uptake. Also, rhizobacteria application had a positive effect on the leaf Mg content only in half of the combinations. The highest positive effect was in Granny Smith (4.0% for N) and Jeromine (4.4% for Ca) cultivars on the M.9 rootstock, and Scarlet Spur (14.1% for P), Fuji (7.1% for K), and Granny Smith (14.0% for Mg) cultivars on the MM.106 rootstock. The highest positive effect for Fe and Mn contents was observed in the Jeromine (12.0%) and Red Chief (9.6%) cultivars budded on MM.106 rootstock, respectively. While the highest effect in the leaf Zn contents was achieved with 7.1% (Scarlet Spur/MM.106) and 7.0% (Golden Reinders/M.9), the rhizobacteria effect on leaf Cu contents reached the highest level of 9.0% and 8.2% in the combinations of both M.9 rootstock and MM.106 rootstock with the Galaxy Gala cultivar, respectively. The highest positive effect of rhizobacteria on the leaf B contents varied between 2.3 and 3.1% in scion–rootstock combinations. On the other hand, the rhizobacteria did not have a positive effect on micronutrient uptake in some scion–rootstock combinations, for example, in combinations of Golden Reinders/M.9 for Fe and Cu uptake, Fuji/MM.106 for Mn uptake, and Granny Smith/MM.106 for Zn uptake.

Table 3. Contribution of rhizobacteria application on leaf nutrients in apple scion–rootstock combinations (%).

	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu	B
Rootstock										
M.9	1.3a	4.2b	1.4b	0.9	1.3	2.7b	3.8	3.8	1.8b	1.3a
MM.106	0.7b	6.6a	3.8a	1.0	1.4	5.1a	3.8	2.9	3.4a	0.3b
Cultivar										
Scarlet Spur	0.4abc	7.4ab	4.5a	−1.7de	0.4b	5.8ab	1.1c	4.5ab	2.4bc	1.8
Fuji	−0.3bc	10.7a	4.2a	−5.2e	2.2a	1.8cd	2.6bc	1.0c	1.0cd	−0.7
Granny Smith	2.9a	4.2bc	1.5ab	8.6a	0.4b	−2.0d	2.2bc	1.3c	4.0b	−0.5
Galaxy Gala	1.3abc	5.6abc	2.1a	−2.8de	0.6b	6.3a	7.8a	2.4bc	8.6a	0.6
Golden Reinders	2.2ab	7.4ab	−1.8b	0.4cd	1.6a	2.4bc	3.0bc	6.8a	0.4cd	2.0
Red Chief	1.6abc	0.8c	4.2a	2.4bc	1.9a	5.2abc	4.4bc	5.3ab	−0.7d	2.1
Jeromine	−0.8c	1.9c	3.2a	5.2ab	2.3a	7.8a	5.3ab	2.4bc	2.6bc	0.4
Scion–rootstock combination										
Scarlet S./M.9	3.1abc	0.8de	3.7bcd	0.0cdef	−1.2b	8.0ab	−1.0fg	1.9bcde	1.6cd	2.8a
Fuji/M.9	−1.0cd	9.1abcd	1.4d	−5.2f	3.1ab	1.1cef	8.3ab	1.6cde	1.0cde	0.1abc
G.Smith/M.9	4.0a	3.5cde	1.2d	3.1bcd	0.2ab	−2.4ef	4.6abcde	6.6ab	1.5cd	−2.0c

G.Gala/M.9	0.8abcd	10.1abc	0.7d	−1.4def	0.5ab	6.0abc	8.7ab	0.5def	9.0a	2.5a
Golden R./M.9	0.9abcd	8.2abcd	−1.2e	−2.1def	0.0ab	−3.8f	1.3cdefg	7.0a	−2.7e	2.3a
Red Chief/M.9	2.6abc	−1.4e	1.7d	5.1bc	2.2ab	6.3abc	−0.9efg	4.4abcde	−1.2de	3.1a
Jeromine/M.9	−1.0cd	−0.6e	2.1cd	7.2b	4.4a	3.5bcde	5.5abc	4.9abcd	3.3bc	0.3abc
Scarlet/MM.106	−2.4d	14.1a	5.3bc	−3.5ef	2.0ab	3.5bcde	3.3bcdef	7.1a	3.2bc	0.7abc
Fuji/MM.106	0.3abcd	12.3ab	7.1a	−5.2f	1.3ab	2.5bcdef	−3.1g	0.5def	1.0cde	−1.5bc
G.Smith/MM.106	1.9abcd	5.0bcde	1.9d	14.0a	0.7ab	−1.5def	−0.2defg	−3.9f	6.5ab	1.0abc
G.Gala/MM.106	1.9abcd	1.1de	3.6bcd	−4.2f	0.6ab	6.7abc	6.9ab	4.2abcde	8.2a	−1.3bc
Golden/MM.106	3.5ab	6.7abcde	−2.4e	3.0bcde	3.2ab	8.6ab	4.7abcd	6.6ab	3.4bc	1.8ab
R.Chief/MM.106	0.7abcd	2.9cde	6.7ab	−0.4cef	1.7ab	4.1bcd	9.6a	6.2abc	−0.2cde	1.1abc
Jeromine/MM.106	−0.7bcd	4.4bcde	4.2bc	3.2bcd	0.3ab	12.0a	5.0abcd	−0.1ef	2.0cd	0.6abc

The difference between the averages indicated by different letters in the same column, separately, for rootstock, cultivar, and scion–rootstock combination is significant ($p < 0.05$). The absence of letters indicates no statistical significance.

3.2. Fruit Nutrient Contents

The fruit nutrient contents differences among the cultivars (except for Ca element) and scion–rootstock combinations in the control group were statistically significant, but were not for rootstocks, except for N, Mn, and Cu contents (Table 4). Considering rootstock effects on the nutrient content of the fruits, the cultivars on the MM.106 rootstock in N content, and the cultivars on the M.9 rootstock in Mn and Cu elements had higher values. Considering the cultivar effect, the Red Chief cultivar showed the highest statistical values in five elements' content (P, K, Mg, Cu and B). It was observed that the fruit N contents ranged from 0.22% (Golden Reinders/M.9) to 0.50% (Jeromine/MM.106) in the scion–rootstock combinations. The highest fruit P, K, and Mg contents were observed in the Red Chief/M.9 combination with 0.32 mg/kg, 9.87 mg/kg, and 1.10 mg/kg, respectively, and the lowest values were obtained in the Golden Reinders/MM.106 combination with 0.14 mg/kg, 6.78 mg/kg, and 0.85 mg/kg, respectively. On the other hand, the fruit Ca contents were the highest in Galaxy Gala/M.9 (3.89 mg/kg) and the lowest in Granny Smith/MM.106 (3.21 mg/kg). Fruit Fe contents varied between 7.62 and 12.15 mg/kg according to the combinations; Galaxy Gala and Golden Reinders cultivars budded on MM.106; and the Red Chief cultivar budded on M.9 had the highest values. The highest fruit Mn contents were observed in the Granny Smith/M.9 combination with 1.66 mg/kg, and the lowest value was obtained in the Fuji/MM.106 combination with 0.41 mg/kg. Some combinations had the highest value in fruit Zn, Cu, and B contents, but the Scarlet Spur/M.9 among scion–rootstock combinations stands out in these contents. On the other hand, the fruit Mn, Zn, Cu, and B contents were at the lowest level in the Fuji/MM.106 combination.

Table 4. Effect of rootstocks, cultivars, and scion–rootstock combinations on fruit nutrients in the control application.

	N	**P**	**K**	**Mg**	**Ca**	**Fe**	**Mn**	**Zn**	**Cu**	**B**
	% (dw)					**mg/kg (dw)**				
Rootstock										
M.9	0.30b	0.24	8.69	1.04	3.59	10.60	1.30a	15.42	1.93a	7.87
MM.106	0.43a	0.21	7.89	0.97	3.49	10.40	0.82b	14.87	1.69b	7.17
Cultivar										
Scarlet Spur	0.39ab	0.23c	8.64b	1.03ab	3.52	11.00c	0.98c	16.90b	1.87a	10.78a

Fuji	0.41a	0.20d	7.31d	1.02ab	3.41	8.87d	0.49d	13.00g	1.54b	2.94e
Granny Smith	0.34d	0.26b	8.58b	0.96bc	3.35	11.30b	1.35a	15.20d	2.02a	9.02b
Galaxy Gala	0.37bcd	0.21d	8.18c	1.03ab	3.67	12.04a	1.23b	17.23a	1.85a	6.65c
Golden Reinders	0.29e	0.16e	7.17d	0.92c	3.43	11.36b	1.01c	13.41f	1.56b	5.06d
Red Chief	0.35cd	0.28a	9.08a	1.06a	3.73	11.01c	1.15b	14.21e	1.92a	10.73a
Jeromine	0.39abc	0.23c	9.10a	1.04a	3.65	7.91e	1.21b	16.07c	1.91a	9.34b
Scion–rootstock combination										
Scarlet S./M.9	0.37cd	0.24cde	8.29e	1.05ab	3.61bcd	11.27cd	1.21de	17.61a	2.05a	11.69a
Fuji/M.9	0.40bcd	0.22de	7.78f	1.03ab	3.38de	8.63ı	0.57h	14.49f	1.65cde	3.48ı
G.Smith/M.9	0.24gh	0.29b	8.79d	1.02ab	3.49cd	11.60bc	1.66a	12.62h	2.09a	8.35f
G.Gala/M.9	0.31ef	0.22de	9.39b	1.06ab	3.89a	11.93ab	1.43bc	16.72b	2.11a	6.88g
Golden R./M.9	0.22h	0.18f	7.56f	0.99bc	3.50cd	10.57f	1.41bc	15.51de	1.69bcde	6.25h
Red Chief/M.9	0.27fg	0.32a	9.87a	1.10a	3.60bcd	12.02a	1.49b	15.04e	1.90abcd	9.10e
Jeromine/M.9	0.28fg	0.22de	9.20bc	1.05ab	3.64abc	8.20j	1.33cd	15.95cd	1.99ab	9.34de
Scarlet/MM.106	0.41bcd	0.22de	8.99cd	1.02ab	3.43cde	10.74ef	0.76g	16.20c	1.69bcde	9.87c
Fuji/MM.106	0.43bc	0.19f	6.84g	1.00abc	3.44cde	9.12h	0.41ı	11.51ı	1.43e	2.41j
G.Smith/MM.106	0.45ab	0.22de	8.37e	0.90cd	3.21e	11.00de	1.04f	17.78a	1.94abc	9.70cd
G.Gala/MM.106	0.42bc	0.19f	6.98g	0.99bc	3.45cde	12.15a	1.03f	17.75a	1.58de	6.43gh
Golden/MM.106	0.36de	0.14g	6.78g	0.85d	3.37de	12.15a	0.62h	11.31ı	1.44e	3.87ı
R.Chief/MM.106	0.43b	0.25c	8.29e	1.02ab	3.85ab	10.00g	0.81g	13.37g	1.94abcd	12.36a
Jeromine/MM.106	0.50a	0.24cde	9.01cd	1.04ab	3.66abc	7.62k	1.10ef	16.19c	1.84abcd	9.34de

The difference between the averages indicated by different letters in the same column, separately, for rootstock, cultivar, and scion–rootstock combination is significant ($p < 0.05$). The absence of letters indicates no statistical significance.

The results of the rhizobacteria effect on the fruit nutrient contents in apple cultivars budded on different rootstocks are shown in Table 5. The rhizobacteria effect on these contents was statistically significant in rootstocks, cultivars, and scion–rootstock combinations, and was generally positive. The effects of bacteria on the fruit nutrient contents were higher on the M.9 rootstock for N and Fe nutrients, and on the MM.106 rootstock for other nutrients.

Bacteria application regarding the cultivars had the highest effect in Granny Smith (N, Mg, and Ca elements) and Scarlet Spur (P, Cu and B elements) cultivars, with an increase in the content of three nutrients. Bacteria application made the highest contribution to the nutrient content of the fruits with an increase of 32.1% in the Mn content of the Fuji cultivar. The positive effect reached the highest level in Granny Smith/M.9 (4.9% for N), Jeromine/MM.106 (10.3% for P contents), Golden Reinders/MM.106 (7.9% and 13.3% in K and Mg contents, respectively), and Granny Smith/MM.106 (14.0% for Mg contents) combinations. On the other hand, the rhizobacteria had no effect on fruit macronutrient contents in some scion–rootstock combinations, for example, in five combinations for Mg contents, and in four combinations for N and Ca contents. The rhizobacteria did not have a positive effect on fruit micronutrient contents in some scion–rootstock combinations, for example, in four combinations for Zn contents, and in three combinations for Fe contents. The highest positive effect for Fe and Cu contents was observed in the Red Chief (9.5%) and Scarlet Spur (24.3%) cultivars budded on M.9 rootstock, respectively. The rhizobacteria effect on Mn, Zn, and B contents reached the highest level 41.8% (Fuji/MM.106), 14.3% (Red Chief/MM.106), and 35.4% (Scarlet Spur/MM.106), respectively. On the other hand, bacterial application showed different results compared to scion–rootstock combinations

in increasing or decreasing the nutrient content of leaves and fruits. For example, in the Granny Smith cultivar, leaf Mn and Zn contents increased on the M.9 rootstock but decreased on the MM.106 rootstock. Again, the leaf Mg, Fe, and Cu contents of the Golden Reinders cultivar and the leaf P and Mn contents of the Red Chief cultivar increased on the MM.106 rootstock, while they decreased on the M.9 rootstock. Similar examples are seen in the increase in fruit P, K, Mg, and Ca contents in the Galaxy Gala cultivar and in fruit K and Mg contents in the Red Chief cultivar on MM.106.

Table 5. Contribution of rhizobacteria application on fruit nutrients in apple scion–rootstock combinations (%).

	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu	B
Rootstock										
M.9	1.3a	2.6b	1.4b	1.7b	1.7b	3.3a	9.6b	1.5b	11.9b	10.5b
MM.106	0.9b	5.0a	2.8a	4.8a	3.2a	2.6b	14.1a	6.6a	14.2a	15.0a
Cultivar										
Scarlet Spur	0.9c	8.0a	2.2d	0.1e	0.8d	5.6b	11.3c	-2.3f	22.2a	27.9a
Fuji	−1.6e	5.4c	2.4d	−1.5g	5.7b	−1.0f	32.1a	4.2cd	7.7f	26.1b
Granny Smith	3.6a	0.4f	3.2c	10.8a	9.6a	1.2e	6.7d	2.9d	10.2d	−0.8g
Galaxy Gala	1.2c	−0.9g	−1.6e	−1.1f	−2.0f	3.8d	5.3e	−1.2ef	8.5e	8.8d
Golden Reinders	2.1b	4.8d	5.4a	9.8b	3.6c	4.7c	0.1f	6.6bcd	20.5b	14.3c
Red Chief	2.0b	1.6e	−1.8e	1.0d	−1.2e	8.5a	13.7b	13.6a	13.3c	7.4e
Jeromine	−0.4d	7.4b	5.1b	3.9c	0.6d	−1.9g	13.6b	9.6b	8.6e	5.5f
Scion–rootstock combination										
Scarlet S./M.9	3.3c	8.5b	3.8d	2.6cd	1.8e	7.8ab	14.7d	−2.9h	24.3a	20.5b
Fuji/M.9	−1.8h	3.2f	3.0e	−2.5fg	6.8b	−1.0g	22.4b	4.7e	4.7f	18.5bc
G.Smith/M.9	4.9a	−1.6ı	3.7d	6.8b	5.7c	0.0g	10.6de	2.4ef	13.3d	0.1gh
G.Gala/M.9	0.0f	−4.0j	−4.9j	−3.5g	−5.7j	5.1b–d	3.4fg	−0.1fg	4.8f	5.7ef
Golden R./M.9	0.0f	3.4f	3.0e	6.4b	1.5f	6.2b	−1.3h	3.7e	18.2c	12.8cd
Red Chief/M.9	2.4d	4.1e	−3.9ı	−0.9ef	0.8g	9.5a	8.4def	12.9ab	13.7d	7.6e
Jeromine/M.9	0.0f	4.5e	5.4b	3.3cd	1.2f	−4.3h	8.7de	7.3d	4.1f	8.0e
Scarlet/MM.106	−1.6h	7.6c	0.7g	−2.3fg	−0.3h	3.4de	7.8ef	−1.8fgh	20.2bc	35.4a
Fuji/MM.106	−1.5h	7.6c	1.8f	−0.5ef	4.7d	−1.0g	41.8a	3.7e	10.7d	33.6a
G.Smith/MM.106	2.3d	2.4g	2.7e	14.8a	13.5a	2.3ef	2.7fg	3.4e	7.2ef	−1.7h
G.Gala/MM.106	2.4d	2.2g	1.7f	1.3de	1.8e	2.4ef	7.2ef	−2.4gh	12.3d	11.8d
Golden/MM.106	4.2b	6.2d	7.9a	13.3a	5.7c	3.2de	1.4gh	9.5cd	22.9ab	15.8c
R.Chief/MM.106	1.5e	−0.8h	0.3h	2.9cd	−3.2ı	7.5ab	19.0bc	14.3a	12.9d	7.2e
Jeromine/MM.106	−0.8g	10.3a	4.8c	4.4bc	−0.1h	0.5fg	18.6c	11.8bc	13.2d	3.1fg

The difference between the averages indicated by different letters in the same column, separately, for rootstock, cultivar, and scion–rootstock combination is significant ($p < 0.05$). The absence of letters indicates no statistical significance.

4. Discussion

Since leaf analysis is generally preferred for evaluating the nutritional status of an orchard, yearly programmed leaf analysis could be useful to assess and select best possible rootstocks for any specific ecological condition [31]. For this reason, the evaluation was made mostly on the results obtained from the leaves. The nutrient uptake by plants in

modern apple cultivation could be different depending on the use of rootstock, changing growth ecology and application of biological control agents. Our research has also shown a positive effect of rhizobacteria application on the nutrition content of apple trees, except for Mg content. Since phosphorus anions react rapidly with soil components, when removed from soil solution, these cations do not react with $H_2PO_4^-$ and HPO_4^{2-}, and, therefore, the anions are bioavailable to plants [32]. Our results agreed with the highest increases in P amounts in the leaves, indicating that there was a contribution of bacterial application. These results suggested that *Bacillus megaterium* M3 increased the fixation of phosphorus higher than the increase to the nitrogen fixation with *Azospirillum sp*-245. This observation suggests that there could be some reasons for the lack of plant reaction to increased contents of available N in soil. The genetic differences of rootstock and cultivar have a significant effect on the nutrient content of plants [19]. The difference in plant nutrient uptake between rootstocks may be associated with factors such as root spreading area, capillary root density, root cation exchange capacity, and root secretions. It is also known that the root system plays a major role in the uptake of water and nutrients from the soil and their transport within the plant [33,34]. According to this study, the phosphorus content in the leaves was inversely correlated with the nitrogen content. The lowest phosphorus content was found in the leaves of the trees with the highest nitrogen content. Treder (2022) demonstrated the phenomenon of a marked increase in phosphorus content with a decrease in nitrogen content in non-fertilized apple trees [35]. Treder (2003), too, had shown a decrease in the phosphorus content of apple leaves with an increase in nitrogen fertilization rates [36].

Different researchers have found that the vigor of the rootstock has a significant impact on the uptake of scion nutrient status in apples [37,38]. Amiri et al. (2008) reported that dwarf rootstock may be one of possible causes of mineral deficiencies in apples [39]. A few researchers have reported that scion leaves of trees on more vigorous rootstocks have higher mineral concentrations such as K, Ca, and Mg than those on the dwarf rootstocks [40,41]. In our study, the M.9 rootstock was less efficient than other rootstocks in the absorption of K, Ca, and Mg nutrients from the soil (Table 2). It can be concluded that the reduction in uptake capacity was related in apple dwarfing rootstocks to their smaller root system, and to the graft union that shows very convoluted xylem vessels that act as filters, influencing the balance of different solutes reaching its scion [38]. K concentrations in both leaf and fruit tissues, and fruit Ca and fruit Mg concentrations on the MM.106 rootstock increased significantly with bacterial application (Table 3, 5). Rhizobacteria had efficiency in these nutrients' uptake by plants on the MM.106 rootstock. On the other hand, trees on the M.9 rootstock were more efficient in N, Fe, and Mn uptake (Table 2). Similar results reported that trees on the M.9 rootstock were more efficient in these nutrients' uptake [41–43]. The high levels of elements such as N, Fe, and Mn in the tissues of the cultivars grafted on the M9 rootstock may suggest that this rootstock is more efficient in the uptake and transport of these elements. The higher leaf mineral concentration in the trees on the M.9 rootstock can be explained by its generally lower vegetative growth and yield per tree than the MM.106 rootstock. Rhizobacteria had efficient N and Fe uptake by trees on the M.9 rootstock but did have an Mn uptake. Indeed, it was confirmed by the increase in leaf N, fruit N and fruit Fe concentrations on this rootstock (Table 3 and 5). Enriching the soil with the rhizobacteria inoculum significantly increased the concentrations of many nutrients in apple leaf and fruit tissues. The uptake and transport of minerals in plants may vary under different environmental conditions in relation to rootstock and scion interactions. The effects of rootstocks on mineral utilization efficiency are still unclear. Each rootstock exhibits a range of size control potential and may show a different potential for transporting raw sap (mineral content) from root to leaf [44]. The observed differences in the rates of nutrient displacement between roots and scion in trees can be attributed to the ability of the root system to take up minerals [41].

The objective of this study was to investigate the effects of rhizobacteria application on leaf and fruit nutrient contents in different apple scion-rootstock combinations. In previous studies on apples, similar results have been reported that rhizobacteria applications generally have a positive effect on the leaf nutrient contents. Pirlak et al. [45] determined that rhizobacteria applications (*Pseudomonas putida* BA-8 and *Bacillus subtilis* OSU-142) increased the leaf nutrient contents in Starkrimson and Granny Smith cultivars, but not the Mg element, between 7.9% and 69.2%. Karlidag et al. [46] reported that the leaf nutrient contents of the Granny Smith cultivar increased between 0.6 and 42.5% with rhizobacteria applications (*Bacillus* M3, *Bacillus* OSU-142 and *Microbacterium* FS01), except for the Cu element. Arıkan [47] determined that rhizobacteria applications (*Bacillus subtilis* EY2, *Bacillus atrophaeus* EY6, *Bacillus spharicus* GC subgroup B EY30, *Staphylococcus kloosii* EY37, and *Kocuria erythromyxa* EY43) had increases up to 66.2% in the leaf nutrient contents of the Fuji cultivar budded on the M.9 rootstock, but not for Mg content. The higher leaf nutrient content increases were reported from research according to our rhizobacteria contribution findings with maximum increases up to 10.7%. On the contrary, it was reported that the rhizobacteria had no effect on N, K, Ca, Mn, and Cu uptake, and had a low increase in Mg, Fe, and Zn uptake (between 0.1–1.7%) in five different apple cultivars budded on the MM.106 rootstock [48]. These results may be explained by the difference in the plant nutrient uptake by rootstocks and differences in transmitting them to scions. In reports presented on different fruit species, it was been noticed that rhizobacteria applications had generally positive effects on leaf nutrient contents [49–56].

These findings in the present study were supported by several previous studies on fruit nutrient contents. Treder et al. [35] found an increase in N, Ca, Mg, S, and some microelement concentrations because of bacterial application (*Bacillus* sp., *Bacillus amyloliquefaciens,* and *Paenibacillus polymyxa*) together with chemical fertilization in apple trees. It was stated that significant increases were observed in the amount of most nutrient elements (except Mg and B elements) in apple trees to which mineral fertilization was not applied, and only microbial fertilizer was applied. Kurek et al. [57] reported that the amount of P, K, and Ca in plant leaves increased significantly because of the increase in the number of phosphate solvent microorganisms (*Pseudomonas luteola* BN0834) in the soil, or their application to the soil. This positive effect of rhizobacteria on nutrient contents in fruits and leaves may be explained their ability to have nitrogen fixation, phosphate-solubilizing and produce growth-promoting substances, and to stimulate the availability of Fe, Zn, Cu, and Mn by decreasing the pH of the soil. On the other hand, in the study, bacterial application showed different results compared to scion–rootstock combinations in increasing or decreasing the nutrient content of leaves and fruits. These results related to the nutrient content of leaves and fruits, which can be explained by the difference in how rootstocks benefit from plant nutrients in the soil and reflect them on grafted cultivars.

5. Conclusions

The rhizobacteria effects on these contents were statistically significant in rootstocks, cultivars, and scion–rootstock combinations. While the effects of cultivars and scion–rootstock combinations on leaf nutrients emerged more clearly than rootstocks, rootstocks were as effective as other variables in fruit nutrients. The positive effect of bacteria application on rootstocks was higher on the M.9 rootstock for leaf N and B elements and fruit N and Fe elements, and on the MM.106 rootstock for other elements compared to other rootstocks. When considered based on cultivars, bacteria application increased three nutrients on leaves by more than 5% and made the most positive contribution in Galaxy Gala and Jeromine cultivars. The rhizobacteria application in scion–rootstock combinations had a generally positive effect on the leaf nutrient contents, except for Mg content. This increase in nutrient contents ranged from 3.1% (for B) to 14.1% (for P). Also, the rhizobacteria application increased fruit nutrient contents between 4.9% (for the N element) and 41.8% (for the Mn element). This increase may be explained by the fact that bacteria in the

rhizosphere affect the availability of essential elements, and that they provide to plants. On the other hand, the leaf and fruit nutrient contents and the effect of rhizobacteria application on them differed according to scion–rootstock combinations. In conclusion, nitrogen + phosphorus solvent rhizobacteria applications were found to have great potential for increasing nutrient contents in apples, as in many other crops previously tested.

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