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Effects of Plant Growth Promoting Rhizobacteria on Growth, Yield and Fruit Quality of Pomegranate (*Punica granatum* L.)

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ABSTRACT

This study was carried out in a commercial pomegranate garden in Denizli, in 2017-2018 in order to determine the effects of *Pseudomonas sp. HV 5* and *Micrococcus luteus GC- subgroup B MFDV3* bacterial strains, which are potentially capable of improving plant yield and development, on plant growth, yield and quality. The effects of bacterial application on shoot diameter, fruit width, fruit size, fruit juice content, pH, acidity and yield per tree were statistically in significant but the effects on fruit weight, fruit volume and fruit juice C vitamins were found to be significant. Concomitant use of HV5 and MFDV3 bacteria resulted in increased fruit weight, volume, and amount of vitamin C in fruit juice compared to control. According to the results of the research, it was concluded that the bacterial strains used had a positive effect on fruit juice and vitamin C only, so that different application forms and doses should be tested.

1. Introduction

Turkey is one of the rare countries where a combination of many types of fruit grown in the world. At present, Turkey is one of the most important producers of many fruit species besides being one of the most production of some fruit species in the world.

Pomegranate, Myrtiflora of the family Punicaceae family, the only genus is Punica. The most important species of this genus is *Punica granatum*. Pomegranate (*Punica granatum* L.) is one of the oldest known fruit species known to humans for 6500 years and is considered a source of healing and healing. It covers the regions of Iran, India, Afghanistan, Anatolia, South Asia, Near East, Middle East and South Caucasus (İkinci, 2007). Pomegranate is a plant which is resistant to arid climate conditions, can adapt to different soil structure in a short time and gives regular product every year. Turkey has a suitable ecology for the cultivation of pomegranate as well as many plant species. World pomegranate cultivation in India, Iran and Turkey are among the first three. Pomegranate production in Turkey has increased very rapidly in recent years. Production, which was 60,000 tons in 2002, reached 465,000 tons in 2016 and 559,000 tons in 2019 (TUİK, 2020). Pomegranate, although grown in almost every region in Turkey, especially in the Aegean and Mediterranean coastline and is cultivated widely in Southeast Anatolia (Özgüven and Yilmaz, 2000). Pomegranate production in Turkey is the highest in Antalya, Muğla, Mersin, Denizli and Adana (TUİK 2020). Turkey, because it is within the boundaries of the homeland pomegranate, shows the richness greatly varieties and forms. There are 48 registered and three pomegranate pomegranate cultivars except for the local types currently grown in Turkey (Anon., 2012).

As a result of the expansion of the pomegranate planting areas, pomegranate production has increased significantly and Turkey pomegranate export has increased significantly. Pomegranate exports, which were $ 9.4 million in 2005, increased by more than 10 times as of 2013 and reached 112 million $. In Turkey among the countries where the pomegranate exports, primarily Russia, Ukraine, Germany, Moldova, including Iraq, Romania, Latvia, Croatia and there are some other countries with Bosnia and Herzegovina (Anon., 2020).

The consumption of pomegranate juice is in the form of pomegranate syrup and its fruits are consumed fresh. In addition, the interest in pomegranate production is increasing all over the world in recent years as it has different usage areas such as tannin, pectin, vinegar, dye and ink raw materials, oil, animal feed and obtaining various pharmaceutical raw materials (Tümer, 2006).

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Pomegranate is used in the treatment of various diseases. It has been reported that pomegranate juice and seed oil prolong life and prevent heart diseases and cancer. Pomegranate juice is good for palpitations. Recently, it has been investigated whether pomegranate juice can be used to combat prostate cancer. In addition, it has been reported in recent years that it is a class of foods used in the treatment of AIDS and is one of the nine plants in Japanese patented drugs (Lansky et al., 1998).

The edible portion of the pomegranate is the seed and its surroundings, this region constitutes 52% of the total fruit, 78% of it is fruit juice and 22% is seed. Juice contains 84.5% water and important amounts of water soluble dry matter, sugar, anthocyanins, phenolics, ascorbic acid and protein (Tümer, 2006). Pomegranate is an anthocyanin rich source. Some compounds found in pomegranate juice, seeds and peel are given as follows (Kim et al., 2002).

There is a search for increasing the amount of production and quality due to the increase in interest of pomegranate. The use of chemical fertilizers and pesticides in fruit growing areas is very limited in a significant part of our country. In organic production, the use of biofertilizers (microbial fertilizers) is becoming widespread. The majority of the bioagens used for this purpose live in the root zone of the plants under the soil. For this reason, these microorganisms are usually used in single-year plants and seeds are seeded with microorganisms. The determination of the effect of microorganisms that can live in sub-soil parts of plants on perennial fruit species and determining formulations that can be transferred to practice are very important for the development of modern and organic fruit cultivation. The aim of this study is to determine the effects of some biological agents (*Pseudomonas* sp. HV5 and *Micrococcus luteus* GC-subgroup B (MFDV3) on pomegranate yield and quality, which can potentially increase plant yield and development.

2. Materials and Methods

This study was carried out in Irlıganlı neighborhood of Denizli province between 2017-2018. Hicaz pomegranate cultivars were used as plant material at the age of 6 years.

The soil analysis results of the orchard are given in Table 1. According to the results of the analysis, the amount of organic matter and nitrogen was found to be insufficient. The soil pH was slightly alkaline 8.2. The amount of soil lime was higher.

Table 1
Some physical and chemical properties of the orchard soil (30-60 cm).

Soil parameters	
pH (1:2.5 s:w)	8.2
EC (µS cm^{-1}; 1:5 s:w)	0.45
Organic matter (%)	2.37
Lime (%)	28.8
Clay (%)	55
Silt (%)	44
Sand (%)	1
N (%)	0.12
P (ppm)	17.08
K (ppm)	244
Ca (ppm)	4.23
Mg (ppm)	831
Na (ppm)	170
Fe (ppm)	12.81
Zn (ppm)	2.02
Cu (ppm)	1.02
Mn (ppm)	10.73
B (ppm)	2.21

Hicaznar pomegranate cultivar with pomegranate berries have a small variety. Its efficiency is very high. Fruit weight is 350 g and fruit width is 91 mm. Fruit shell color is 95% red on yellow background. The grains are dark red in color and 100 grain weight average is 26.1g. It has a tasteful taste close to the sour. It grows well in the coastal and passage regions of the Mediterranean Region (Onur, 1983).

Pseudomonas sp. HV5 It was isolated from volcanic soil in Iğdır. The diagnosis of the bacterium was made with the MIDI system used for bacterial characterization. The Diagnostic Sim Index is 38% and is a gram negative strain. Phosphate dissolving and nitrogen fixation is strongly positive.

Micrococcus luteus GC- subgroup MFDV3 Iğdır Aralık district was isolated from salty soils. Diagnosis of bacteria was done by MIDI system. Diagnosis Sim Index is 84% and gram positive bacteria. 7.5% NaCl in the medium is a strain that can live.

The bacteria used were obtained from Iğdır University Faculty of Agriculture. Bacterial breeds were planted on Nutrient Agar and stored at 30°C for 24 hours. At the end of this period, the suspension was prepared in 0.1 M phosphate buffer from bacterial cultures. After the bacterial concentration was set at 10^9 CFU / ml, bacterial suspensions and control application were applied to the crown trace of the trees 3 times in 2 months intervals (Eşitken et al., 2006).

The bacterial applications were planned as randomized plots with 3 replications and 5 trees were used in each replication.

Applications

1. Control

2. HV5; 1 liter was applied to each tree's crown projection by adding 13 liters of water to the bacterial suspension produced as 2 liters. This application was made every two years in April, June and August.

3. MFDV3; 1 liter was applied to each tree's crown projection by adding 13 liters of water to the bacterial suspension produced as 2 liters. This application was made every two years in April, June and August.

4. HV5 + MFDV3; 1 liter of each bacterial suspension and 13 liters of water were added to each tree's crown projection was applied 1 liter each. This application was made every two years in April, June and August.

In this study, plant growth, yield and fruit quality characteristics were examined as follows:

Fruit weight (g): The average weight of 10 fruits selected from the ripe fruits harvested from each tree is weighed on the balance with 0.01 sensitivity.

Fruit volume (cm^3): The volume of 10 fruits selected from each tree is calculated based on the volume of the overflow water.

Measurements of fruit width and length (mm) were made in the largest part of the fruit in the equatorial region of the fruit, with 10 fruits randomly selected from each tree.

Fruit Skin Elasticity (Newton) The elasticity of the fruits was measured by Shoremeter. A 5 mm (0.2 cm) probe was used in the measurement. 1991 Shoremeter ark values between 1-100 shore and 1 Shore = 0.1Newton arasında (Ağar et al., 1991).

The length and diameter of shoots from each tree were measured in mm with the digital caliper.

The amount of total soluble solids (TSS): It was determined as % of the fruits selected from each application by hand refractometer from fruit juice (Yetim, 2001).

Titratable acidity (%): The juice sample was titrated with 0.1 N NaOH to calculate the amount of malic acid (Yetim, 2001).

Vitamin C (mg / 100g) The ascorbic acid content in the samples was determined by spectrophotometric dichlorophenol indophenol method (Pearson, 1976).

The pH of the juice was determined by pH meter.

Yield per plant: The fruits collected from each tree at harvest time were weighed and yield per tree was calculated.

3. Results and Discussion

Results

Length and Diameter of Shoot

The effect of bacterial applications on shoot length was not statistically significant in 2017. In 2018, applications increased the shoot length slightly, but the highest increase was in the implementation of HV5 and MFDV3. In 2018, the length of the shoot was 10.91 mm and it increased to 12.59 mm in the application of 12.18 mm HV5 + MFDV3 in 12.22 mm MFDV3 in HV5 (Table 2). The data obtained are in accordance with the literature in general. Eşitken et al. (2002) stated that OSU-142 bacteria applied to apricot increases shoot length and diameter. At the same time, Eşitken et al., (2006) in their study of BA-8, OSU-142, BA-8 + OSU-142 applications have determined that the length of the shoot increases. Granny Smith apple variety Karlıdağ et al. (2007) in their study in Malatya, the inoculated *Bacillus* M3, *Bacillus* OSU-142 and *Microbacterium* FS01 bacteria were determined to increase the length and diameter of shoots in trees significantly. In another study, 4 bacterial strains regulating plant growth (*Agrobacterium rubi* A-18, *Bacillus subtilis* OSU-142, *Burkholderia gladioli* OSU-7 and *Pseudomonas putida* BA-8) were grafted on MM-106 rootstock. Bacterial applications in the Starkspur Golden Delicious and Golden Delicious apple varieties increased the number of annual shoots and their diameter (Karakurt and Aslantaş, 2010).

Table 2
Effect of bacterial applications on shoot length and diameter

	Shoot Length (cm)		Shoot Diameter (cm)	
	2017	2018	2017	2018
Control	11.34	10.91 b*	3.56	3.46 b
MFDV3	11.63	12.18 a	3.59	3.58 a
HV5	11.45	12.22 a	3.59	3.56 a
MFDV3 + HV5	11.67	12.59 a	3.57	3.54 ab
	N.S.**		N.S.	

*: Means given within columns followed by a different letter are significantly different at P = 0.05
**: Non-significant

Fruit Properties

The effects of the bacterial applications on fruit width were statistically insignificant in 2017 and significant in 2018. In 2018, the highest increase in all fruit cultivars was found in HV5 application with 9.85 cm. In fruit length, there was no significant difference in the application groups in 2017 and 2018 (Table 3). Similarly, Pırlak et al., (2007) in cherry and Eşitken et al. (2006) in apple found that the effect of bacterial applications on fruit diameter was not statistically significant.

The results of the effects of applications on fruit weight are given in Table 3. In 2017, the applications of HV5 and MFDV3 bacteria decreased the fruit weight compared to the control, while the application of these bacteria resulted in an increase in fruit weight. In addition, all applications in 2018 have increased fruit weight. The effect of bacteria on increasing the fruit weight can be attributed to the properties of nitrogen fixation and phosphate removal. Rizobacteria that increase plant growth increase fruit weight in many plant species. As a matter of fact, *Pseudomonas* BA-8 and *Bacillus* OSU-142 bacteria were found to increase plant growth, yield, trunk cross-sectional area, shoot length and fruit weight significantly in the 0900 Ziraat sweet cherry cultivar (Eşitken et al., 2006). In a study carried out in Karaman, *Pseudomonas* BA-8 and *Bacillus* OSU-142 bacterial strains were found to have fruit weight in Starkrimson and Granny Smith apple cultivars (Pırlak et al., 2007). Karlıdağ et al., (2007) in a study conducted in Malatya, *Bacillus* M3, *Bacillus* OSU-142 and *Microbacterium* FS01 radically inoculated bacteria Granny Smith apple varieties have determined that the weight of fruit increases. *Bacillus subtilis* BEB-13bs, which promotes inoculated plant growth in tomato roots, has been found to increase fruit weight (Mena-Violante and Olalde-Portugal, 2007). İpek et al. (2009) found that *Alcaligenes* 637Ca, *Staphylococcus* MFDCa-1, *Staphylococcus* MFDCa-2, *Agrobacterium* A18, *Panteo* FF1 and *Bacillus* M3 bacterial strains, which are compatible with calcareous environments, increased the average fruit weight in Aromas strawberry cultivar by 17.7% compared to control. Shamseldin et al., (2010) in his study of orange Pseudomanas fluorescence 843 strain was used and fruit yield as well as fruit weight was significantly increased compared to the control. İpek et al., (2014) in his study on strawberry *Alcaligenes* 637Ca fruit weight was increased by 9.4% compared to the control.

Table 3
Effect of bacteria on some fruit properties

	Fruit Weight (g)		Fruit Width (cm)		Fruit Length (cm)		Fruit Volume (cm^3)	
	2017	2018	2017	2018	2017	2018	2017	2018
Control	460.49 b*	404.30 b	9.98	9.49 b	8.56	8.23	498.12	408.33 c
MFDV3	431.47 c	436.08 a	9.89	9.72 ab	8.36	8.43	497.11	455.67 ab
HV5	447.47 bc	433.68 a	9.95	9.85 a	8.49	8.32	492.44	432.67 bc
MFDV3+V5	498.48 a	445.42 a	10.27	9.82 a	8.77	8.32	501.32	464.00 a
			N.S.**		N.S.	N.S.	N.S.	

Yield

According to the control of the fruit weight per tree, in terms of yield in 2017 and 2018, no difference was observed according to the control (Table 4.).

Table 4
Yield per tree

	Yield (kg/plant)	
	2017	2018
Control	39.27	40.30
MFDV3	39.63	40.24
HV5	39.48	40.15
MFDV3 + HV5	40.22	40.66
	N.S.*	N.S.

*: Non-significant

In this case, it cannot be mentioned that bacterial applications affect the number of fruits and fruit weight in a positive way. When the yield was compared between 2017 and 2018, no significant difference was observed in both control and application groups. In a study performed by Eşitken et al. (2009), there was no significant effect of OSU-142 bacteria application on yield in Golden Delicious apple. In another study, *Azotobacter chroococcum* nitrogen fusion bacteria and *Glomus mosseae* mushroom combination were used and six years of pomegranate plants showed a significant improvement in the yield of the crops under field conditions (Mir and Sharma, 2012). İpek et al., (2014), *Alcaligenes* 637Ca bacterial strains increased the fruit yield by 47.5% compared to the control in strawberry. Erturk et al., (2012) RC19 (*Bacillus simplex*), RC05 (*Paenibacillus polymyxa*) and RC23 (*Bacillus* spp.) Was radically inoculated and as a result, a significant increase in efficiency compared to the control was observed in Fern strawbeery cultivar.

Fruit chemical properties

Bacterial applications do not have a significant effect on the TSS (Table 5). Ipek et al., 2014 in his study of strawberry fruit in the study of rhizobacteria has not seen a significant effect on TSS. Similarly, Eşitken et al., (2006) cherry and Orhan et al. (2006) in raspberry studies on the effects of bacterial applications were found to be insignificant.

In the study, it was found that HV5, MFDV3 and HV5 + MFDV3 applications did not have a significant effect on the fruit juice pH (Table 5).

Fruit juice titratable acidity decreased as a result of the applications, but this decrease was not statistically significant (Table 5). The results are in parallel with the literature. Eşitken et al., (2006) in cherry, Orhan et al., (2006) in raspberry and Pırlak and Köse (2010) in their study of strawberries bacterial applications on the amount of titratable acidity was found to be insignificant.

As a result of the application of HV5, the amount of vitamin C was found to be the same as the control application, while the amount of vitamin C was increased in combination with the application of MFDV3 and bacteria (Table 4.4). The most important source of vitamin C, a water-soluble vitamin, is fresh fruits and vegetables and this increase can be considered as positive. Shamseldin et al., (2010) in their study using *Pseudomanas fluorescence* 843 strain Washington Orange vitamin C concentration was not changed in the first year, but when applied in the second year showed a significant increase compared to control. Ordookhani et al. (2013) reported that an increase in the concentration of vitamin C was induced by the application of plant growth promoting bacteria (*Pseudomonas putida* strain 41, *Azotobacter chroococcum* strain 5 and *Azosprillum lipoferum* strain) to the tomato plant. Erturk et al., (2012) Fern strawberry cultivar RC19 (*Bacillus simplex*), RC05 (*Paenibacillus polymyxa*) and RC23 (*Bacillus* spp.) As a result of the inoculation of vitamin C amount of control according to the control increased with the application of all three rhizobacteria.

Table 5
Effect of Bacterial Applications on Some Fruit Chemical Properties

	TSS (%)		pH		Titratable Acidity (%)		Vitamin C (mg/100g)	
	2017	2018	2017	2018	2017	2018	2017	2018
Control	16.63	16.25	3.12	3.13	5.74	5.65	0.45 b	0.47 b
MFDV3	16.00	16.08	3.11	3.09	4.62	4.74	0.45 b	0.48 b
HV5	15.93	15.85	3.16	3.14	4.23	4.34	0.50 a	0.52 a
MFDV3 + HV5	16.50	16.77	3.13	3.11	4.36	4.29	0.56 a	0.55 a
	N.S.**	N.S.	N.S.	N.S.	N.S.	N.S.		

*: Means given within columns followed by a different letter are significantly different at P = 0.05
**: Non-significant

Fruit Skin and Grain Color

The effects of applications on fruit skin and grain color are given in Table 6. The fruit skin color was determined by Minolta Konica CR-400 cromometer and L, C and Hue values were calculated. L value is the value indicating the brightness of the fruit and this value does not differ according to the control in the MFDV3 and HV5 application. Skin color C value showed significant difference with MFDV3 application. There is no statistical difference in the other groups. Hue value as a result of HV5, MFDV3 and HV5 + MFDV3 applications decreased in all 3 groups. The lowest value according to the control was found in HV5 application with 47.49.

When we look at the results of fruit grain color, L, C and Hue values were determined. Accordingly, L and C values decreased statistically significantly after MFDV3 application. The most serious reduction in C was observed in the combination of bacteria. The Hue value has decreased at the maximum HV5 application.

Table 6
Effect of Bacterial Applications on Fruit Skin and Grain Color

	Skin Color						Grain Color					
	2016			2017			2016			2017		
	L	C	Hue	L	C	Hue	L	C	Hue	L	C	Hue
Control	61.85a	42.06b	58.26a	63.43a	41.31b	52.27a	22.87b	22.15a	23.55ab	23.31b	21.97a	23.28a
MFDV3	60.18	42.55b	46.25d	59.50a	42.97b	47.49d	24.19ab	22.48a	22.31c	24.02ab	22.37a	22.38cb
HV5	63.58a	46.85a	51.44c	63.39a	45.65a	52.40c	21.67c	19.85b	22.95bc	21.45c	18.23b	22.59bc
MFDV3 + HV5	54.55b	40.98b	54.92b	53.89b	41.24b	55.61b	25.24a	15.69c	24.05a	24.51a	14.78c	23.86a

*: Means given within columns followed by a different letter are significantly different at P = 0.05
**: Non-significant

4. Conclusion

In this study, we investigated the effects of HV5 and MFDV3 biochemicals on the growth, fruit yield and properties of pomegranate. As a result of the applications, there are no serious positive and negative effects, and this is due to the fact that the applications do not reach the root area completely. Because, after some applications, sufficient irrigation could not be done, so the bacteria could not reach the root area in full. In addition, the deep rooted roots of the pomegranate plant, which is generally rooted due to irrigation insufficiency, may have prevented the bacteria from reaching. As a result, significant effects in general have emerged in the co-administration of both biosystems. Based on this, it was concluded that more detailed studies on these two bacterial strains as well as bacterial strains that stimulate different growth to be used in organic production in pomegranate plants should be tried.

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