

Bacillus atrophaeus MFDV2 RHIZOBACTERIA ISOLATE INCREASES VEGETATIVE GROWTH, YIELD, AND FRUIT SIZE OF BANANA PLANT

Isolat Rhizobakteri *Bacillus atrophaeus* MFDV2 Meningkatkan Pertumbuhan Vegetatif, Hasil, dan Ukuran Buah Tanaman Pisang

Yaşar Akbaş^a, Lütfi Pirlak^{a*}, and Mesude Figen Dönmez^b

^a Department of Horticulture, Faculty of Agriculture, Selçuk University,
Selçuklu, 42079, Konya-Turkey

^b Department of Plant Protection, Faculty of Agriculture, Iğdır University,
Iğdır-Turkey

*Corresponding author: lpirlak@hotmail.com

Submitted 17 October 2019; Revised 19 December 2019; Accepted 26 December 2019

ABSTRACT

Plant growth-promoting rhizobacteria have important roles in soil fertility and have been manipulated for ecologically friendly crop production. The study aimed to determine the effects of a biological agent (*Bacillus atrophaeus* MFDV2) on the growth, yield, and quality of the banana plant. The study was conducted in 2017–2018 using the banana Dwarf Cavendish clone. Bacterial isolate was cultured on nutrient agar and stored at 30 °C for 24 hours. The culture was then suspended in 0.1 M phosphate buffer to 10⁹ CFU ml⁻¹. The bacterial suspension was applied as foliar, soil and foliar + soil once every two months starting from February. One liter of suspension was applied on the soil and over the leaves per plant. The results showed that the effect of bacterial treatment did not increase stem circumference and leaf length, but it was a significant increase in plant height, leaf number, and leaf width. The effects of bacterial application on banana bunch weight and fruit growth were statistically significant. All applications significantly increased the weight, with the highest increase occurring in foliar finger + soil application. While the effects on the finger diameter were statistically similar. Application on leaf + root has been the biggest increase in finger length. The effects of bacterial applications increased N, P, and K contents in the leaves. The study concluded that the application of *Bacillus atrophaeus* MFDV2 increased the vegetative growth and fruit quality of the banana plant, so it is advisable to be used in organic banana farming.

[**Keywords:** Plant growth-promoting rhizobacteria, banana, organic farming]

ABSTRAK

Rhizobakteri pemacu pertumbuhan tanaman memiliki peran penting dalam kesuburan tanah dan telah dimanipulasi untuk produksi tanaman yang ramah lingkungan. Penelitian ini bertujuan untuk mengetahui efek agen biologis (*Bacillus atrophaeus* MFDV2) terhadap pertumbuhan, hasil, dan kualitas buah tanaman pisang. Penelitian dilakukan pada 2017-2018 dengan menggunakan klon pisang Dwarf Cavendish. Isolat bakteri dikultur pada medium nutrient agar dan disimpan pada suhu 30 °C selama 24 jam. Kultur kemudian disuspensikan dalam 0,1 M bufer fosfat menjadi 10⁹ CFU ml⁻¹. Suspensi bakteri

diaplikasikan pada daun, tanah, dan daun + tanah setiap dua bulan mulai Februari. Satu liter suspensi diaplikasikan di tanah dan di atas daun setiap tanaman. Hasil penelitian menunjukkan bahwa perlakuan bakteri tidak meningkatkan lingkaran batang dan panjang daun, tetapi secara signifikan meningkatkan tinggi tanaman, jumlah daun, dan lebar daun. Efek aplikasi bakteri pada bobot tandan pisang dan pertumbuhan buah secara statistik signifikan. Semua aplikasi nyata meningkatkan bobot buah, dengan peningkatan tertinggi terjadi pada aplikasi daun + tanah. Sementara efek pada diameter buah secara statistik serupa. Efek aplikasi rizobakteri pada daun + tanah nyata meningkatkan panjang buah. Aplikasi rhizobakteri meningkatkan kandungan N, P, dan K dalam daun. Studi ini menyimpulkan aplikasi *Bacillus atrophaeus* MFDV2 meningkatkan pertumbuhan vegetatif dan kualitas buah tanaman pisang sehingga disarankan untuk digunakan dalam budi daya pisang secara organik.

[**Kata kunci:** Rhizobakteri pemacu pertumbuhan tanaman, pisang, pertanian organik]

INTRODUCTION

Turkey is one of the rare countries where a combination of many types of fruit is grown in the world. Turkey is one of the fruit production centers in the world. Bananas have an important place among these fruit species. The banana was brought from Egypt and planted by families who were interested in Egypt for the first time in 1750 as an ornamental plant in Alanya, Turkey. In those years, when the banana was grown as an ornamental plant in Alanya, it was started to be grown for commercial purposes for fruit after the 1930s (Mendilcioğlu 1997). After this date, the production of bananas in our country increased rapidly. In 1955, the production of which was 1,070 tons rose to 369,009 tons in 2017 (TSI 2019).

Agriculture is one of the most important livelihoods of hundreds of thousands of farming families in Turkey who earn their living from the fruit culture. However, the

per unit area yield per tree or fruit production in Turkey is not at the desired level. The most important way to increase efficiency is the implementation of cultural measures as necessary.

Organic and artificial fertilizers are the most commonly used substances to increase the yield in plants. Especially the use of artificial fertilizers is increasing rapidly. However, there are various problems related to the use of these fertilizers. Therefore, studies are carried out for the application of various organic substances as fertilizers.

Various commercially produced chemicals are also used to increase plant yield and to combat plant diseases. Against the chemicals used caused the negative effects on microorganisms such as resistance to environment, environmental pollution, negative effects on plant, animal and human health and expensive are an alternative to the use of beneficial microorganisms (Avis et al. 2008). Besides, chemical fertilizer application methods as well as biological agents, have been increasing in recent years to increase yield and fruit quality.

The use of bioagents is essential for organic farming. The majority of bioagents used for this purpose live in the root region of plants. For this reason, these microorganisms are usually used in single-year plants and as seed treatment. Determining the effect of the microorganisms that can live in the sub-parts of plants on perennial fruit species and formulations that can be transferred to the practice is very important for the development of modern and organic fruit cultivation. In light of this information, the study aimed to determine the effects of a biological agent on the growth, yield, and quality of the banana plant. The study is expected to minimize the use of chemical fertilizers hence increase plant growth and yield.

MATERIALS AND METHODS

Experimental Site

The research was carried out in the banana garden of the Dwarf Cavendish clone, which was built on a 4000 m² area in Antalya Province between 2017-2018. The garden is located at an altitude of 134 m above sea level and at 36 ° 26'34 "North - 32 ° 09'28" East coordinates.

Analysis results of soil samples taken from the experimental land are given in Table 1. Accordingly, soil pH was 7.57, the amount of lime was 2.84%, EC (mS) was 2060, and organic matter was found to be 3.6%.

Bacterial Multiplication

Bacillus atrophaeus MFDV2 strain used in the study was obtained from the Agricultural Faculty of Iğdır

Table 1. Soil properties of the experimental banana garden in Antalya Province, Turkey.

Soil properties	Value	Category
pH (1:2.5)	7.57	Light alkaline
Lime (%)	2.84	Little
EC (mS)	2060	Little salty
Sand (%)	68	
Clay (%)	0	
Silt (%)	0	
Organic matter (%)	3.6	Good
P (ppm) (Olsen)	300	Much
K (ppm)	370	Much
Available P2O5 (kg da ⁻¹)	171.86	
Available K2O (kg da ⁻¹)	111.46	

University. The strain was isolated from volcanic soil in Iğdır and strongly fixed nitrogen and dissolved phosphate.

The bacterial isolate was cultivated 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 (Sigma-Aldrich Code P4417 included sodium phosphate, sodium chloride, potassium chloride, and potassium phosphate) from bacterial cultures.

Experimental Design and Bacterial Application

The experiment was designed as a completely randomized, three replications and three plants were used in each replication. The treatments were control, foliar application, soil application, and foliar + soil application.

The bacterial suspension, adjusted to 10⁹ CFU ml⁻¹ concentration, was applied once every two months on the soil and leaves from February (Arikan and Pirlak 2016). Application from the soil is made homogeneously to the crown projection of the banana plants. One liter of bacterial suspension was applied per plant. In foliar application, the prepared solution was sprayed to the plants leaves. Bacterial applications were carried out in the evening in cool time for the effect of absorption and application on the above ground. Bacterial applications were performed on 3 February, 15 April, and 17 June 2017.

Data Collection

The stem diameters of the plants were determined by using the caliper at 20 cm of the soil surface at the time of the bunch occurrence. The plant length was determined from the soil level at the time of the crest formation by measuring with the tape meter. For a total number of leaves, all of the leaves were counted at the time of the emergence of the leaves. The length and width of the

three leaves selected from each plant were determined by measuring them with tape. The weight of each cluster in plants was determined by weighing at harvest time. For finger weight in hand (g), after harvesting and ripening, the fruits were determined by weighing in the sensitive scales five fingers taken from the lower and upper rows of the first, middle, and last hand. Finger circles were measured using calipers and finger lengths were determined by measuring with tape meter After harvesting the fruits of the cultivated plants, the contents of the TSS were determined by hand refractometer (Gübbük et al. 1993).

For plant nutrient analysis, samples taken from the fully developed leaves of the plant were dried in the oven. P, K, Ca, Mg, Fe, Mn, Zn, Cu, B and S contents were analyzed in three different steps with nitric acid-hydrogen peroxide (2:3) mixture (step 1, 75% at 145 °C at microwave power 5 minutes; step 2, 90 minutes at 180 °C, microwave power at 10 minutes, and step 3 at 100 °C in 40% microwave power for 10 minutes) at 40 bar pressure-resistant microwave at the burner unit after reading on the spectrophotometer, while N content was determined by micro-Kjeldahl method (Mertens 2005).

Data Analyses

Data obtained from the study were subjected by analysis of variance (ANOVA) and Duncan's multiple range tests separated means.

RESULTS AND DISCUSSION

Banana Growth

The effects of bacterial applications on plant growth are given in Table 2. Accordingly effects of the applications on the stem diameter and leaf length were statistically

insignificant, while the effects on plant height, leaf number, and leaf width were found to be significant.

Foliar, soil, and foliar + soil bacterial applications increased the number of leaves in plants. The average number of leaves per plant was 11.51 in control, 13.14 from foliar, 13.29 from soil, and 13.42 from foliar + soil application. In general, the presence of healthy leaves and photosynthesis increases on the surface of the plant have a positive effect on plant development. Similarly, bacterial applications also increased leaf width in banana plants. The highest increase occurred in the fundamental application. The leaf width of 82.24 cm was observed in control and 94.98 cm in soil application with an increase of 15%. The bacterial application also significantly increased in plant height and the highest increase was obtained from soil application. The average plant height of 2.92 m was observed in control plants, 3.06 m in foliar application, 3.10 m in foliar + soil application, and 3.13 m in soil application. The growth-promoting synthesis of rhizobacteria can explain the effects of this bacterium, which promotes plant growth and increases leaf number, leaf width and plant height.

The bacteria used in our study also have the feature of nitrogen fixation and phosphate removal. It has been reported that rhizobacterium strains that promote plant growth significantly increase vegetative growth in studies conducted previously in different fruit species. Karakurt et al. (2013) examined the effects of some bacterial strains (*Pantoea agglomerans* strain RK-79, RK-80 and RK-92, *Serratia liquefaciens* strain RK-102 and *Pseudomonas putida* strain RK-142) on the growth of apricot. All bacterial strains caused significant increases in the number of annual exiles, shoot size, and shoot length. Pirlak and Köse (2010) *Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M3 bacterial strains Selva and Sweet Charlie strawberry varieties in their study to determine the effects on quality and yield of seedlings per plant number of plants per plant, leaf area and can be used significantly have found that they have increased. Eşitken et al. (2006) reported that the application of *Pseudomonas* BA-8 and *Bacillus* OSU-142 bacteria on 0900 Ziraat cherry cultivar significantly increased yield, body cross-sectional area, shoot length, and fruit weight. In India the use of the nitrogen-fixing *Azotobacter*, *Azospirillum* and *Beijerinckia* bacteria alone and in combinations on mulberries increase leaf area and quality Sudhakar et al. (2000). *Pseudomonas fluorescens* (Pf5, PRA25, 105, 101), *Bacillus pumilus* (T4), *Pseudomonas corrugata* (114) and fungal isolates *Gliocladium virens* (G1-21) and *Trichoderma harzianum* (T22) promotes plant growth in blueberries and increased stem diameter and leaf area (De Silva et al. 2000). Pirlak et al. (2007) in a study using *Pseudomonas* BA-8 and

Table 2. The effects of bacterial applications on the growth of bananas.

	Stem diameter (cm)	Leaf number	Leaf length (m)	Leaf width (cm)	Plant height (m)
Control	20.71	11.51b*	2.47	82.24c	2.92c
Foliar application	21.56	13.14a	2.61	91.08b	3.06b
Soil application	21.09	13.29a	2.64	94.98a	3.13a
Foliar + soil application	20.96	13.42a	2.54	91.11b	3.10ab
LSD	NS**	0.61	NS	1.99	0.10

*: Mean separation within columns by Duncan's multiple tests at 0.05 level.

**: Non-significant

Bacillus OSU-142 bacterial strains on Starkrimson and Granny Smith apple varieties, have determined that they increase stem cross-sectional area, annual shoot length and diameter, and leaf area compared to control. Turan et al. (2014) reported that using *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92, and *Bacillus subtilis* TV-17C bacterial strains in the form of seed coating on cabbage seedlings increased root weight, body dry weight, leaf area, trunk diameter, seedling growth, and quality parameters such as seedling growth rate and improved chlorophyll content.

PGPR treatments on different plants improved morphological features, physiological parameters, biochemical properties, and plant nutrition. In these studies, plant height, shoot length, shoot diameter, root length, root number, fresh and dry plant weight, fresh and dry root weight, stem diameter, leaf area, fruit yield, and germination rates were promoted by PGPR applications (Abd El-Daim et al. 2014; Delshadi et al. 2017; Hou et al. 2018; Hussain et al. 2018). Chlorophyll content, antioxidant enzyme activity, non-enzymatic antioxidant content, proline content, protein content, membrane permeability, stomatal conductance, photosynthetic activity, amino acid content, organic acid content, plant growth regulators, and leaf relative water content (LRWC) have been affected positively by PGPR treatments (Hussain et al. 2018; Kakar et al. 2016; Kumar et al. 2016).

Effects of Bacterial Applications on Fruit Properties

Banana plants form a single bunch of flowers throughout their lifetime, and after this bunch becomes a fruit and matures, the plant's life ends. As such, the bunch weight also means yield per plant. Therefore, the large bunches increase the yield and thus the income on the unit area. The combs on the banana bunches and the fingers on the combs, that is, the banana fruits occur (Mendilcioglu 1997).

The effects of applications on bunch weight and fruit size were found to be statistically significant. The application of bacterial bacteria in the soil increased bunch weight according to the control, and no significant change was observed in the other applications compared to the control. The average cluster weight, which was 41.26 kg in control, increased to 46.78 kg with an increase of 13.4%. All of the applications increased the finger weight significantly compared to the control, and the most increase occurred in the foliar + soil application. With this application, finger weight increased by 23% compared to control.

Similarly, applications increased finger diameter and finger length compared to control. While the effects of the applications on finger diameter were found to be

statistically similar, the application which increased the finger length most was foliar + soil. The effects of bacterial applications on TSS in fruits were not statistically significant (Table 3).

Bacterial application to increase the yield and fruit size of the banana increased the number and size of leaves, hence increased the photosynthesis surface. It can be explained by the fact that bacteria increase the synthesis of growth-regulating agents and stimulate the intake of nutrients from the soil. As a matter of fact, in many studies conducted on different plant species about the subject, it has been reported that growth-enhancing bacterial applications, in general, increase the yield and improve fruit properties. *Alcaligenes* 637Ca bacterium application on Aromas strawberry cultivar increased yield per plant by 47% compared to control and increased average fruit weight by 17.7% (İpek et al. 2009). In studies investigating the effects of different root bacteria on the yield of tomato plants grown in perlite in the greenhouse, *Bacillus* spp. strain 66/3 caused an increase in yield by 37% in autumn and 18% in spring compared to control (Kıdoğlu 2009). In the case of vaccination with sugar beet, the root and leaf weight, and sugar yield increased. Starking Delicious, Granny Smith, Starkrimson Delicious, Starkspur Golden Delicious and Golden Delicious apple varieties grafted on MM-106 rootstocks were used to determine the specific gravity of fruit, pit depth of fruit stem and application of A-18, OSU-142, OSU-7 and BA-8 bacteria increased yield per tree (Pırlak et al. 2007). In a pomegranate garden in Denizli province, *Pseudomonas syringae* pv. *syringae* HV5 and *Micrococcus luteus* GC-subgroup B with MFDV3, which can increase the plant yield and has a development potential, has been investigated to determine the effects of bacterial strains on plant growth, yield, and quality (Acar 2018). The effects of bacterial applications on shoot length, shoot diameter,

Table 3. Effects of bacterial applications on fruit properties in bananas.

Treatment	Bunch weight (kg)	Finger weight (g)	Finger length (cm)	Finger diameter (cm)	TSS (%)
Control	41.26b*	135.0c	22.12c	3.11b	21.02
Foliar application	43.71b	146.1b	23.54b	3.42a	21.08
Soil application	46.78a	149.1b	23.58b	3.36a	21.20
Foliar + soil application	43.27b	166.2a	24.68a	3.52a	21.11
LSD	3.7	5.46	1.03	3.11	N.S.**

*: Mean separation within columns by Duncan's multiple tests at 0.05 level.

**: Non-significant

Table 4. Effect of bacterial application on nutrient contents of banana leaves.

Treatment	N (%)*	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)
Control	2.97b	3264.0b	20680.9b	8193.0a	2845.5a	139.9a	38.85a	36.89a	16.17c
Foliar application	3.02b	3194.3b	19508.2c	7782.8c	2764.3b	120.0d	37.46b	37.07a	17.22b
Soil application	2.99b	3228.5b	19544.8c	7932.6b	2877.5a	127.3c	36.30c	37.29a	17.42b
Foliar + soil application	3.16a	3509.0a	21276.5a	7875.4c	2837.1a	128.7b	34.21b	34.21b	18.52a

*: Mean separation within columns by Duncan's multiple tests at 0.05 level.

fruit width, fruit size, fruit juice, TSS, pH and acidity, and yield per tree were statistically insignificant, while fruit weight, fruit volume, and fruit juice effects on vitamin C were found to be important. As a result of the combination of HV5 and MFDV3 bacteria, fruit weight, volume, and amount of vitamin C in fruit juice increased compared to the control.

Effect of Bacteria on Plant Nutrient Element Contents of Leaves

The effects of bacterial applications on leaf nutrient content were found to be statistically significant (Table 4). As a result of applications, the most significant increase in N content compared to control occurred in foliar + soil application. Nitrogen, 2.97% in control, increased to 3.16% in foliar + soil application. The most significant increase in leaf P content compared to control occurred in foliar + soil application. P of 3264 mg kg⁻¹ was found as 3509 mg kg⁻¹ in foliar + soil application. K at 20,680 mg kg⁻¹ was found to be 21,276 mg kg⁻¹ in foliar + soil application. Ca, Fe, B and Mn contents in leaf decreased as a result of bacterial applications. In the same application, Mg content of 2845 mg kg⁻¹ in control increase 2,877 mg kg⁻¹ in soil application, and Cu of 18.52 mg kg⁻¹ in control showed a significant increase at 37.29 mg kg⁻¹ in soil application. Egamberdiyeva and Höflich (2004) found that plant growth-promoting bacteria derived from the root region of different agricultural products significantly increased N, P, and K contents in cotton and pea in the semi-arid region of Uzbekistan. *B. megaterium* vaccination was determined to increase P intake in sugar cane (Yadav and Singh 1990).

The researchers reported that PGPR applications decreased the rates of fertilizers such as N, P, and K under stressed and non-stressed conditions (Aras et al. 2018; Arıkan and Pırlak 2017; Esitken et al. 2010; İpek et al. 2017). The decreasing fertilizer use significantly reduces the possibility of the secondary salinity occurrence. Thus, using PGPR would prevent formation of salinity in the soil.

CONCLUSION

The application of plant growth-promoting rhizobacteria (*Bacillus atrophaeus* MFDV2) increased the vegetative growth, yield, and fruit size in the treated banana plants. The bacteria increased N, P, and K contents in the leaves. Therefore, the application of the bacterial isolate can be recommended to reduce the use of N, P, and K fertilizers in conventional cultivation and to meet the nutrient requirements of plants in organic banana cultivation.

ACKNOWLEDGMENTS

This study was supported by Selçuk University Scientific Research Projects Coordination Office with project no. 18201019.

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