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RESEARCH ARTICLE

Effects of PGPR Bacteria Applications on Soil Properties, Plant Growth and Yield Values in Karaerik and Narince Grape Varieties

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that promote plant growth by adhering to the root surfaces in the rhizosphere region of plants. In addition to improving the physical properties of soils, these bacteria increase plant growth and yield by positively affecting nitrogen fixation, phosphorus solubility, water and nutrient uptake of plants. In this study, the effects of bacteria applications on the vegetative development and yield levels of Karaerik and Narince grape varieties, which are important table varieties of Erzincan and Tokat regions, grown in greenhouses in Erzurum central conditions were investigated. In the study, 4 different bacterial combinations (*Pseudomonas chlororaphis* + *Paenibacillus pabuli* + *Bacillus simplex* + *Pseudomonas fluorescens*) that promote plant growth were applied to the plant root zone as a solution. In the study, the effects of PGPR applications on the vegetative growth of vines, some pomological characteristics, yield levels, macronutrient contents of leaves and physical and chemical properties of greenhouse soils were determined. While aggregate stability and porosity values of PGPR treated soils increased, water permeability and bulk density values decreased. Bacterial applications in both grape varieties showed a positive effect on shoot length, shoot diameter, number of nodes, berry width, berry length, cluster width, cluster length, number of seeds, number of clusters, cluster weight, number of berries, berry weight, total yield and macronutrient content of leaves. According to the control group, PGPR applied soils; organic matter content increased by 76.2%, aggregate stability values increased by 49.5% and porosity by 5.5%, while water permeability decreased by 18.3% and bulk density by 3.9%. Depending on the application, it was determined that the yield increased by 42.8% in Karaerik grape variety and 35.7% in Narince grape variety.

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1. Introduction

Soil is an environment that contains various microorganisms and provides habitat for many plants and animals. In recent years, in order to make agricultural production more efficient and more sustainable, biostimulators applied directly to plants or to the rhizosphere are defined as substances or microorganisms that increase nutrient uptake and product quality, reduce the need for fertilizer, and promote plant growth. Microbial biostimulants include mycorrhizal and non-mycorrhizal fungi, bacterial endosymbionts (such as Rhizobium), and plant growth-promoting rhizobacteria (PGPRs). The type and amount of microbial communities in the soil is an important indicator of the soil quality index and is effective in the management of agricultural systems (Elliott et al., 1996; Mäeder et al., 2002; Hoorman 2016). Among these microorganism communities, the bacteria associated with plant

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roots are called root bacteria. Root bacteria are effective in the decomposition and mineralization of organic residues in the soil. In this process, bacteria give to soil an intense biochemical activity and physical quality. This situation affects both the soil structure and other living things in the soil ecosystem, especially plants (Hacımüftüoğlu & Canbolat, 2022).

Many studies have shown that these bacteria living in the rhizosphere support plant growth with different mechanisms of action. Rhizobacteria that support plant growth are also known as “Probiotic Rhizobacteria” due to the many benefits they provide to the plant (John et al., 2020). These bacteria, called PGPR, which inhabit the plant root surface and rhizosphere soil, can promote plant growth through direct and indirect mechanisms of action (İmriz et al., 2014). While some rhizobacteria (Azotobacter, Azospirillum, Beijerrinckia and Pseudomonas i.e.) play a role in nitrogen fixation (Reis et al., 1994), it is reported that some bacteria increase the synthesis of growth-regulating substances in plants (Zahir et al., 2004). Plant growth-promoting PGPR bacteria, through biological fixation and phytohormone production, promote nitrogen fixation and enable the solubilization of phosphorus and heavy metals in the rhizosphere region. These bacteria, in addition to increasing water and mineral uptake by supporting root development, play an important role in biological control of plant diseases and pests (Mayak et al., 2004; Hynes et al., 2008; Berg & Smalla, 2009; Lugtenberg & Kamilova, 2009; Annapurna et al., 2011; El-Boray et al., 2013; Philippot et al., 2013; Panke-Buisse et al., 2015; Tangolar, 2022). Addition of bacteria to soils plays an important role in nutrient cycling in plant development (Elo et al., 2000).

It has been demonstrated in many studies that the germination rate, root and shoot performance, yield, leaf area, chlorophyll ratio, nitrogen ratio, protein ratio, hydraulic activity and drought tolerance in plants increase with PGPR applications (Dobbelaere et al., 2001; Şahin et al., 2004; Altın & Bora, 2005; Dos santos et al., 2020). Although soil and plant nutrition are the most important factors for crop production, intensive fertilization on the same agricultural land for many years threatens the productivity of agricultural lands. Excessive use of fertilizers in order to obtain more products per unit area is increasing day by day, leading to environmental problems and depletion of natural resources. Intensive farming practices can cause water and wind erosion in agricultural areas, depletion of nutrients, loss of soil organic matter and deterioration of various physical properties of the soil (Ruzzi & Aroca, 2015). This situation in crop production requires more production with less input. Plant growth-promoting rhizobacteria (PGPR) are used to minimize fertilizer application and maximize plant development and nutrition (Cakmakci et al., 2006; Sonneveld & Voogt, 2009; Yildirim et al., 2011; Yadav et al., 2015). Studies have revealed that rhizobacteria have significant potential on vegetative and generative development in horticulture plants. Grapes, one of the most widely grown fruits among horticultural crops, have great potential for sustainable agricultural production. The grapevine root system, which is the most important organs involved in water and nutrient uptake and storage, is the first plant part to be affected by soil properties. The development of the above-ground parts of vines is closely related to the structure and health of the root system (Southey, 1992; Smart, 1995). However, the main issue is to reveal the relationship between the targeted yield and quality levels in the vine and root functionality, soil structure and vine performance.

The purpose of this study; to reveal the effects of PGPR bacteria, which have become increasingly used in organic agriculture in recent years, on Karaerik and Narince grape varieties grown under cover (greenhouse) in Erzurum central conditions. Within the scope of this research, depending on the changes caused by PGPR bacterial applications on the physical and chemical properties of greenhouse soils; nutrient intake, vegetative development levels and productivity of grapevines were investigated.

2. Materials and Methods

In the research, 6-year-old vines of Narince and Karaerik varieties grown on their own roots, which are widely grown in Erzincan and Tokat regions, were used. The vineyards grown under unheated greenhouse conditions were given water once a month during the vegetation period. The total of 24 plants were used in the study. While 3 plants were left for the control groups and PGPR bacteria were applied to 9 plants in two different grape varieties in the experiment.

Harvest was performed on September 21, 2022, at the end of the 90th day of bacterial application. Shoot length values were obtained by measuring the length of the summer shoots randomly selected from the vines on the day of harvest, from the point of attachment to an old branch, to the apical. The average shoot diameter values were determined by measuring the diameter between the 2nd and 3rd node of the branch used in the shoot length measurements with a caliper. For each application, the clusters on the vine were counted one by one, before veraison and during the harvest period. The number of clusters determined in two different periods was in harmony with each other. Two clusters were taken from each vine and weighed on a sensitive scale, and the average cluster weight was determined by dividing the total value by the number of clusters. Width and length values of the clusters were determined with a caliper. The number of berry in the bunch was determined by counting the fully ripened berries in 2 harvested clusters. The berries representing the cluster were selected from the middle part of the cluster and the width and height values were determined with a caliper. Berry weight values were determined in grams by weighing 10 berries taken from the clusters of vines on a 0.1g sensitive scale (Gürsöz, 1993). The number of seeds in 10 berries taken from the selected clusters was counted and the average value per berry was determined. The yield value was obtained by multiplying the number of clusters on a vine by the average cluster weight (Kupe & Kose, 2015). In order to reproduce the bacteria, 'Nutrient agar' medium was prepared at the rate of 28 g/L, sterilized and poured into petri dishes. The 'Nutrient Agar' medium was placed in a glass balloon and sterile distilled water was added and made up to 1 L. The media were placed in an autoclave and sterilized at 121 °C for 15 minutes and, it was then poured into petri dishes without solidifying at about 40 °C and left to cool at room temperature (Kızıloğlu & Bilen, 1997). Reproduction was made from bacterial cultures kept as stock and kept at -70 °C, pure culture was cultivated on nutrient agar medium and incubated at 28-30 °C for 5 days (Gürgün & Halkman, 1988). Bacteria prepared after incubation were transferred onto nutrient broth medium sterilized at 121 °C for 15 minutes in order to multiply bacterial cultures. It was incubated in a shaker for approximately 48 hours (50-60 rpm) and made ready for planting (Kızıloğlu & Bilen, 1997). In the study, 4 different bacterial combinations (*Pseudomonas chlororaphis* + *Paenibacillus pabuli* + *Bacillus simplex* + *Pseudomonas fluorescens*) that support plant growth were applied to the root zone of the plant in solution form, adjusted to a concentration of 10^8 CFU. In the study, the effects of PGPR applied on the vegetative growth, some pomological characteristics, yield levels and macronutrient contents of the leaves were determined.

According to USDA (1999), the soils used in the research are in the sandy loam class. Sub-samples to be used for basic analyzes were prepared by sieving through 2 mm sieves from soil samples that were duly taken from the greenhouse and air-dried under laboratory conditions. Soil texture was determined by Bouyoucos hydrometer method (Gee & Bauder, 1986), soil reaction (pH) by glass electrode pH meter (McLean, 1982), lime content by Scheibler calcimeter (Nelson, 1982), organic matter content by Smith Weldon method (Nelson & Sommers, 1982), electrical conductivity (EC) value with electrical conductivity instrument (Rhoades, 1982), aggregate stability (AS) using Yoder type wet sieving device (Kemper & Rosenau, 1986), water permeability (Klute & Dirksen, 1986), particle density were determined by pycnometer method (Blake & Hartge, 1986). On the other hand, soil bulk density by the cylinder method (Blake & Hartge, 1986), total porosity was calculated from bulk weight and particle density, P_2O_5, Ca^{++}, Mg^{++}, Na^+ and K^+ contents of the soils were determined by Kacar (2014)'s method. Statistical analysis was performed by ANOVA, and differences between means were tested using Duncan's multiple range test.

3. Results and Discussion

Basic soil analysis results are presented in Table 1. In this study, the texture class of the soils was determined as coarse textured in the sandy clay loam texture class (62% sand, 30% silt, 8% clay). Soil organic matter content (3.55%) is in the well class; pH level was found to be 7.62 and neutral, the EC level of the working soils is 1.23 dS/m without salt, the $CaCO_3$ level was determined as 5.11% in the medium calcareous (Ülgen & Yurtsever, 1995), class. According to the available phosphorus contents, the class of the soils was determined as medium (Ülgen & Yurtsever, 1995), Ca^{++}, Mg^{++}, Na^+, K+ contents were determined as 8.15, 5.38, 1.28, 2.24 me/100 g, respectively. Particle density was determined as 2.68 (g/cm^3) (Table 1).

Table 1. Results of some basic physical and chemical analyzes of the researched soils.

Soil Properties	
Sand (%)	62
Silt (%)	30
Clay (%)	8
Texture class	Sandy loam
Particle density (g/cm^3)	2.68
Organic matter (%)	3.55
pH	7.62
Electrical Conductivity (dS/m)	1.23
$CaCO_3$ (%)	5.11
Ca, Mg, Na, K (me/100 g)	8.15, 5.38, 1.28, 2.24

Vegetative growth, pomological characteristics and yield values of Karaerik and Narince grape cultivars were found to be statistically higher in plants treated with PGPR bacteria than control groups (Tables 2 and 3).

Table 2. Vegetative development parameters of Karaerik and Narince varieties.

Vegetative development parameters	**Karaerik Variety**		**Narince Variety**	
	Control	**Application**	**Control**	**Application**
Shoot Length (cm)	3.80ab	4.80a	2.50b	3.50ab
Shoot Diameter (cm)	1.40b	1.63a	1.16c	1.30bc
Node Number (item)	28.6ab	34.6a	21.6b	27.3b

When vegetative development parameters were examined, it was determined that there was a statistical difference between the application and control groups ($p<0.05$). When the shoot length parameters were examined, the highest value (4.80 cm) was determined in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was determined that the shoot length value in the Karaerik grape variety increased by 26.3% compared to the control group, and in the Narince grape variety, there was a 40% increase. When the shoot diameters of the vines were examined, it was determined that there was an increase of 16.4% and 12%, respectively, in Karaerik and Narince grape varieties compared to the control groups, depending on the applications. The highest value was determined in the application group of Karaerik grape variety, with 1.63 cm. When the average number of nodes per shoot of vines was examined, it was determined that the number of nodes increased in parallel with the shoot length. It was determined that PGPR bacterial application increased the number of nodes in Karaerik and Narince grape varieties by 21% and 26%, respectively, compared to the control group (Table 2).

Table 3. Pomological features and yield of Karaerik and Narince varieties.

Pomological features and yield	Karaerik Variety		Narince Variety	
	Control	Application	Control	Application
Berry Width (cm)	2.03b	2.16a	1.70d	1.86c
Berry Size (cm)	2.56b	2.70a	1.73d	1.93c
Cluster Width (cm)	14.87b	18.76a	14.06c	15.46b
Cluster Size (cm)	28.3a	29.03a	22.43c	24.46b
Number of Seeds (item)	3ab	3.33a	2c	2.33bc
Number of Clusters (item)	5.33b	6.66a	4.66b	5.33b
Cluster Weight (g)	629.3b	719.3a	367d	435.6c
Number of Berry (item)	66.30b	82.00a	81.00a	81.30a
Berry Weight (g)	7.10b	7.83a	3.00d	4.03c
Yield (g)	3354.17b	4790.5a	1710.2d	2321.7c

When pomological development and yield parameters were examined, it was determined that there was a statistical difference between the application and control groups ($p<0.05$). When the berry width values were examined, the highest value (2.16 cm) was determined in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was determined that there was an increase of 6.40% in the berry width value of the Karaerik grape variety compared to the control group, and a 9.41% increase in the Narince grape variety (Table 3). When the berry size was examined, it was determined that there was an increase of 5.46% and 11.5%, respectively, in Karaerik and Narince grape varieties compared to the control groups, depending on the applications. It was revealed that the highest value was in the application group of Karaerik grape variety, with 2.70 cm (Table 3). When Table 3 was examined, it was determined that there was a parallel relationship between cluster width and cluster length values.

Depending on the PGPR application, it was determined that cluster width and cluster length values increased by 26.2% and 2.58%, respectively, in Karaerik grape variety compared to the control groups, and by 10% and 9.1% in Narince grape variety. When the number of seeds in the berry was examined, it was determined that the highest average value (3.33 items) was in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was determined that the number of seeds in the Karaerik grape variety increased by 11% compared to the control group, and in the Narince grape variety, there was an increase of 16.5% (Table 3). When the number of clusters on the vine was examined, it was determined that there was an increase of 25% and 14.4%, respectively, in Karaerik and Narince grape varieties compared to the control groups. It was determined that the highest average number of clusters was in the application group of the Karaerik grape variety with 6.66 units. When the cluster weight values were examined, the highest value (719.3 g) was determined in the Karaerik grape variety application group. Depending on the PGPR bacterial application, it was determined that there was an increase of 14.3% in the Karaerik grape variety and 18.7% in the Narince grape variety in cluster weight values compared to the control groups. When the number of berries on the cluster was examined depending on the applications, it was determined that there was an increase of 23.7% and 0.37%, respectively, in Karaerik and Narince grape varieties compared to the control groups. It was determined that the highest average number of berries was in the application group of the Karaerik grape variety with 82 item (Table 3). When the berry weight values were examined, the highest value (7.83 g) was determined in the Karaerik grape variety application group. It was determined that there was an increase of 10.3% in Karaerik grape variety and 34.3% in Narince grape variety in berry weight values depending on the treatments compared to the control groups. When the average yields of the vines determined based on the average number of clusters and average cluster weight values were examined, it was determined that the highest yield value was in the Karaerik application group with 4790.5 g. Depending on the applications, it was determined that an increase of 42.8% occurred in Karaerik grape variety compared to the control group. Similarly, depending on the application, it was determined that there was a 35.8% yield increase in Narince grape variety compared to the control group (Table 3).

Table 4. Macro elements contents of Karaerik and Narince leaves.

Macro elements (%)	Karaerik leaves		Narince leaves	
	Control	Application	Control	Application
Ca	0.28b	0.37a	0.27b	0.36a
Mg	0.23c	0.26b	0.23c	0.31a
Na	0.12d	0.15c	0.23b	0.26a
K	1.42c	1.56a	1.40c	1.51b
N	1.44d	1.56c	1.66b	1.69a

When the macronutrient contents of leaves in Kararerik and Narince grape varieties are compared, depending on the PGPR bacterial application, the Ca, Mg, Na, K and N contents in the Karaerik grape variety are 9%, 3%, 3%, 14%, 12%, and in Narince grape variety are increased by 9%, 8%, 3%, 11%, 3% respectively compared to the control groups. When Table 4 was analysed, it was observed that the highest macro element content was generally found in the leaves of Narince grape varieties treated with PGPR.

3.1. Soil Physical and Chemical Properties

At the end of the 90th day of PGPR bacteria combination application, the physical properties were determined on the soil samples taken from the plant root zone. Aggregate stability, water permeability, bulk density and porosity values of the research soils are given in Table 5.

Table 5. Effects of application on the physical and chemical properties of soils.

	Control	Application
AS (%)	41.20	61.60
WP (cm/h)	7.10	5.80
BD (gr/cm^3)	1.54	1.48
Porosity (%)	41.90	44.20
OM (%)	1.43	2.52
pH	7.64	7.78
EC	1.23	1.11
Lime (%)	9.17	9.20
P_2O_5 (kg/da)	6.78	7.45
K_2O (kg/da)	246	246

AS: Aggregate stability, **WP:** Water permeability, **BD:** Bulk density **OM:** Organic matter.

According to the results, it was determined that the PGPR applications applied to the soils had a significant effect on the physical properties of the soils. Rhizobacteria are known to increase microaggregates in soil by binding soil particles together (Ingham, 2009; Hacimuftuoglu, 2020). When aggregate stability and porosity values of bacteria-inoculated soils are examined, it is seen that there is a significant increase compared to the control group soils. According to Table 5, it was determined that aggregate stability and porosity values increased by 49.5% and 5.5%, and water permeability and bulk density values decreased by 18.3% and 3.9%, respectively, in the treated soils compared to the control group. Vandevivere and Baveye (1992) and Abdel Aal et al. (2010) found in their research that the addition of bacteria to soils clogged soil pores and significantly reduced soil permeability, depending on microbial biomass.

When the soil chemical properties were examined, it was determined that the most important effect occurred at the organic matter level. It was determined that the organic matter level, which was 1.43% in the control group, increased to 2.52% in the treated soils with an increase of 76%. Since beneficial bacteria in the soil environment convert organic content into usable nutrients, their presence and activities in the soil are very important (Badalucco & Kuikman, 2001). This situation can positively affect soil aggregation as well as soil productivity (Hayes, 2010; Hoorman, 2016). Development of structure in agricultural lands; is a key factor in soil quality and crop production (Six et al., 2000; Díaz-Zorita et al., 2002; Bronick & Lal, 2005). Soil structure provides soil formation and stabilization by controlling biological activity, plant growth and nutrient cycling (Denis & Caron, 1998). The application of organic matter in the soil provides a more suitable environment for plant growth by providing a positive effect on the soil's aggregate stability, water permeability, air-water balance, and uptake of plant nutrients in the soil (Bronick & Lal, 2005; Ingham, 2009; Hacımüftüoğlu & Küpe, 2022). As a result of the research, it was determined that PGPR bacterial applications increased the organic matter content in soils by increasing bacterial activity and had a positive effect on aggregate stability values. It was determined that PGPR bacterial applications changed the physical properties of the soil, thus positively promoting vegetative and generative development in vines.

When Figure 1 was examined, it was determined that while the aggregate stability and porosity values of the soil increased due to PGPR bacterial applications, the soil bulk density and soil water permeability values decreased. Depending on these changes in soil physical properties, it is seen that the shoot development of the vines also increases.

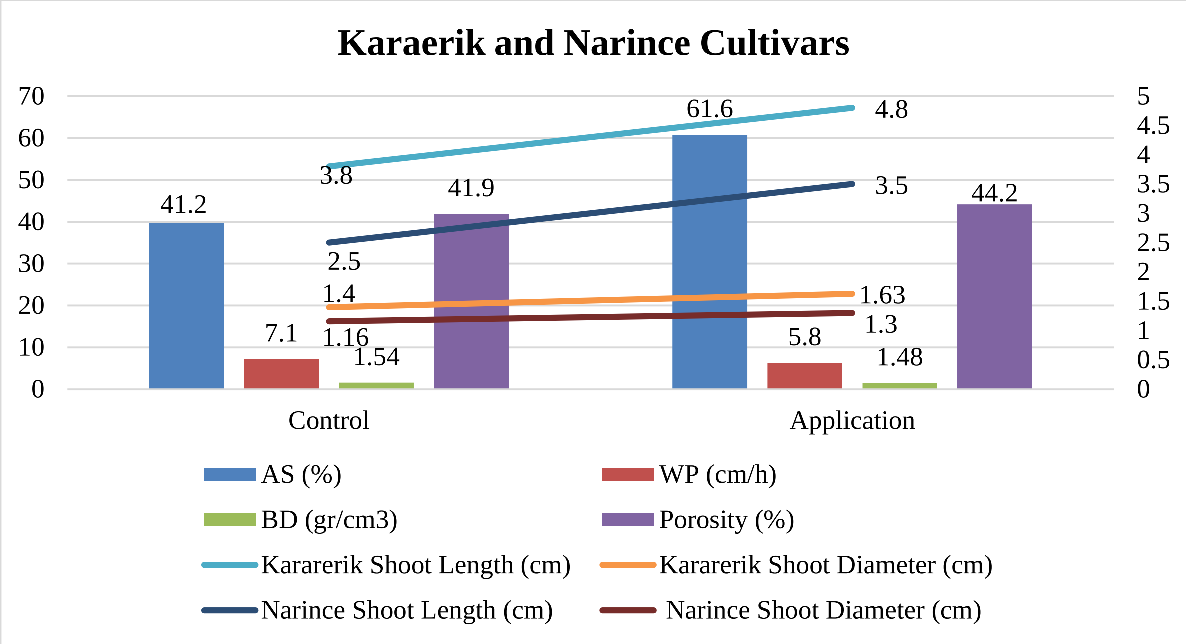

Figure 1. The relationship between soil physical properties and plant vegetative development.

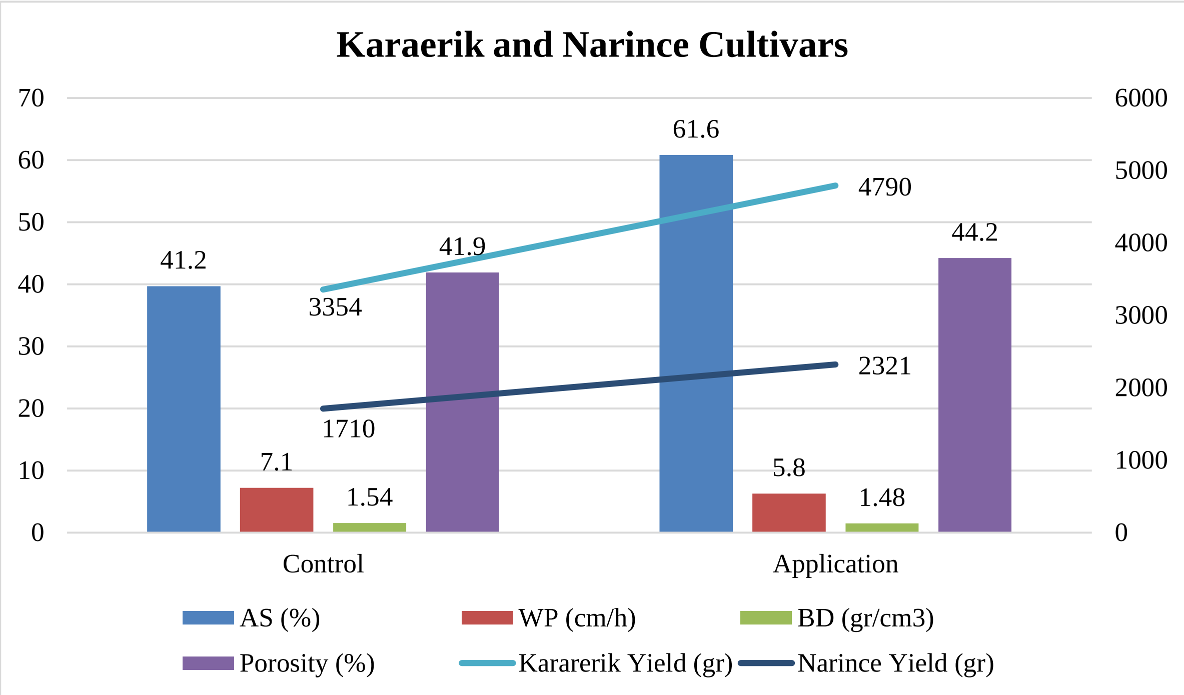

Figure 2. The relationship between soil physical properties and crop yield.

Depending on PGPR bacterial applications, the aggregate stability of soils increased from 41.2% to 61.6%. In parallel with this development, it was determined that the yield value of the Karaerik grape variety increased from 3354 g/vine to 4770 g/vine, and the yield value of the Narince grape variety increased from 1710 grams per vine to 2321 grams (Figure 2).

Soil nutritional status affects all parts of the grapevine, from root growth and distribution through to shoot growth and grape composition (Lanyon et al., 2004). The functional aspects of soil structure, namely water supply (Hamblin, 1986) and aeration (Gupta & Larson, 1982) are the two most important soil characteristics determining suitability of soil for viticulture (Northcote, 1988). These are properties that we need to ascertain their degree of influence on vine performance, with specific attention to root and shoot growth, yield and grape quality. Few studies have been conducted on the effect of soil strength on vine root growth. Among these, Myburgh et al. (1996) found, in an extensive survey of soil conditions in vineyards in all the major grape producing areas across South-eastern and Western Australia, that poor vine performance (either yield or quality) could often be traced to restricted root development. Rowe (1993) and Wang et al. (2001) demonstrated that, even in situations where water and nutrient availability are non-limiting, the size of the root system has a direct effect on shoot growth and, hence, associated vine balance. As a matter of fact, different studies have stated that PGPR bacterial applications in grapevines have positive effects on vegetative development and mineral uptake (Sabir et al., 2012; Gunes et al., 2015; Korkutal et al., 2020). In this study, it was determined that PGPR bacterial applications stimulated root development in the soil of both grape varieties. In parallel with, a positive effect occurred on shoot length, shoot diameter, number of nodes, grain width, grain length, cluster width, cluster length, number of seeds, number of clusters, cluster weight, number of grains, grain weight and total yield values. It has also been observed that it has a positive effect on the macronutrient content of the leaves.

4. Conclusion

It is known that different rhizobacteria activate different mechanisms on yield parameters. In this study, multiple bacterial combinations, which have been shown to be effective on soil fertility in many studies, were applied to soils. The findings revealed that bacterial treatments significantly increased the degree of soil aggregation, plant vegetative growth and yield parameters. These bacteria play a key role in improving the physical properties of soils for plant root growth and continue to be an important part of organic farming activities. In today's world, where the need for access to plant products comes to the forefront, the importance of bacterial applications is becoming increasingly important for the protection of human health.

It has been determined that PGPR bacteria applications have a significant positive effect on vegetative growth and yield parameters of vines. It was determined that the number of clusters and cluster weights increased as well as the shoot growth of the vines applied. In the light of the data obtained in this study, it is thought that more comprehensive studies in viticulture may be useful to reveal the specific activities and physiological mechanisms of PGPR bacteria applied to soils. We believe that the organic viticulture sector will be more efficient and economical with the widespread use of PGPR bacteria applications in vineyards.

Conflict of Interest

The authors declare that they have no conflict of interest.

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