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Effects of PGPR Usage on Yield and Quality of Different Head Lettuce (*Lactuca sativa* var. *capitata*)

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ABSTRACT

This study was conducted at Van Yuzuncu Yil University's Department of Horticulture to evaluate the effects of various PGPR (Plant Growth-Promoting Rhizobacteria) isolates on three lettuce varieties under field and greenhouse conditions. The varieties included Great, Kıvırcık, Iceberg for field conditions, and Chianti, Defne, Bombolo for greenhouse conditions. PGPR isolates used were Control, FZB42 (*Bacillus amyloliquefaciens*), CC44 (*Pseudomonas fluorescens*), and CC37/2 (*Pantoea agglomerans*). Field trials showed Iceberg had the largest head diameter (11.48 cm), and Great had the highest head weight (452.91 g). In greenhouse trials, Bombolo had the largest head diameter (10.91 cm), and CC44 led to the highest head weight (125.22 g). Kıvırcık yielded the highest leaf number in the field, while Bombolo excelled in greenhouse conditions. Although PGPR isolates had limited effects on leaf number, they significantly influenced head diameter and weight, especially in field trials. Variety differences were crucial, with PGPR effects varying based on environmental conditions. Additionally, pH levels were significantly impacted, with Great recording the highest leaf pH in the field and Chianti in the greenhouse. These findings suggest that selecting appropriate PGPR strains and optimizing environmental factors can enhance lettuce yield and quality. Future research should focus on broader field trials and the integration of PGPR with reduced chemical fertilizers for sustainable lettuce production.

Farklı Baş Salata (*Lactuca sativa* var. *capitata*) Çeşitlerinde PGPR Kullanımının Verim ve Kalite Üzerine Etkileri

Makale Bilgisi

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ÖZET

Bu çalışma, Van Yüzüncü Yıl Üniversitesi Bahçe Bitkileri Bölümü'nde, üç marul çeşidinin (Great, Kıvırcık, Iceberg tarla koşullarında; Chianti, Defne, Bombolo sera koşullarında) ve üç farklı PGPR izolatının (Kontrol, FZB42: *Bacillus amyloliquefaciens*, CC44: *Pseudomonas fluorescens*, CC37/2: *Pantoea agglomerans*) büyüme, verim ve kalite üzerindeki etkilerini değerlendirmiştir. Tarla denemelerinde Iceberg en büyük baş çapına (11,48 cm) ve Great en yüksek baş ağırlığına (452,91 g) ulaşmıştır. Sera denemelerinde Bombolo en büyük baş çapını (10,91 cm) ve CC44 en yüksek baş ağırlığını (125,22 g) sağlamıştır. Kıvırcık, tarla koşullarında en fazla yaprak sayısını üretirken, Bombolo sera koşullarında öne çıkmıştır. PGPR izolatları yaprak sayısını sınırlı şekilde etkilemiş, ancak baş çapı ve ağırlığını özellikle tarla koşullarında anlamlı şekilde etkilemiştir. Çeşit farklılıkları belirleyici olmuş, PGPR etkileri çevresel koşullara bağlı olarak değişmiştir. pH seviyeleri de önemli ölçüde etkilenmiş olup, tarla koşullarında Great ve sera koşullarında Chianti en yüksek pH'ı vermiştir. Sonuçlar, uygun PGPR suşlarının seçilmesi ve çevresel faktörlerin optimize edilmesinin verim ve kaliteyi artırabileceğini göstermektedir.

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INTRODUCTION

Lettuce has various types, such as leaf, cos, or head lettuce, and is among the important vegetables of the Compositae (Asteraceae) family. Its leaves are consumed fresh or cooked. In addition to lettuce, other species, such as artichoke and chicory, are also part of this family (Günay, 1981). Worldwide, lettuce is an annual cool-climate vegetable that can be grown in all seasons and has a wide consumer base. In Türkiye, especially head lettuce varieties have seen diversified production and consumption in recent years (Aybak, 2002). Lettuce possesses a deep and extensive root system, making it highly sensitive to soil structure and irrigation patterns. Proper fertilization and water management are crucial for optimizing the yield and quality of head lettuce (Eşiyok et al., 1996). Additionally, lettuce is sensitive to temperature fluctuations, with optimal growth occurring between 15.5 °C and 18.3 °C (Çivit and Akıncı, 2010). Due to favorable climatic conditions, lettuce can be cultivated year-round in various regions of Türkiye (Günay, 2005; Kabay et al., 2018; Küçük et al., 2024).

Plant growth-promoting rhizobacteria (PGPR) colonize plant roots, enhancing seed germination, root development, and water uptake. PGPRs promote plant growth by producing growth hormones and modifying the microbial balance in the rhizosphere. They also protect plants against diseases by suppressing soil borne pathogens (Siddiqui, 2006; Bilge et al., 2019; Tunçtürk et al., 2019). Research conducted in Türkiye has shown that PGPRs positively impact head lettuce and general lettuce cultivation (Kıdoğlu et al., 2007; Akköprü et al., 2018). PGPR applications are particularly effective in supporting plant development in seedlings, leading to a higher success rate (Yan et al., 2003). Studies investigating the effects of PGPRs in organic lettuce production have demonstrated that these bacteria enhance root development and improve plant nutrient uptake. PGPR applications are highlighted as a promising alternative to chemical fertilizers, offering significant potential to support plant growth (Malkoçlu et al., 2016; Çiylez and Eşitken, 2018).

This study aimed to investigate the effects of three PGPR isolates (FZB42: *Bacillus amyloliquefaciens*, CC44: *Pseudomonas fluorescens*, and CC37/2: *Pantoea agglomerans*) on the growth, yield, and quality of head lettuce cultivars, including Great, Kıvırcık, and Iceberg in field conditions, and Chianti, Defne, and Bombolo in greenhouse conditions.

MATERIALS AND METHODS

Materials

The study was conducted in the greenhouses and application area of the Department of Horticulture, Faculty of Agriculture, Van Yuzuncu Yil University. Field and protected cultivation trials were carried out in 36 plots, using a randomized block design with 12 treatments and 3 replications. The experiment aimed to evaluate the effects of three head lettuce varieties and three different PGPR isolates on plant growth, yield, and quality.

The field trial started with seed sowing on April 14, 2016, and measurements were completed on August 12, 2016. In the field trial, plant varieties (Great, Curly, and Iceberg) were grown from seed. In the greenhouse trial, seedlings (Chianti, Laurel, and Bombolo) were purchased due to seed-related problems and time loss. Seedling planting started on October 2, 2016, and measurements were completed on December 15, 2016.

In both trials, beside to the control group 3 PGPR isolates (FZB42: *Bacillus amyloliquefaciens*, CC44: *Pseudomonas fluorescens*, CC37/2: *Pantoea agglomerans*) were applied to the head lettuce. PGPR applications were made to the root zone during seed sowing, seedling emergence and developmental stages in the field and before and after transplanting in the greenhouse.

The soil was cultivated at an appropriate depth and planting density was 40 cm between rows and was 30 cm in rows. There were 12 plants in the field plots and 24 plants in the greenhouse plots. Soil analysis was performed in both application periods and fertilization was carried out on June 7, 2016 in the field trial and on November 4, 2016 in the greenhouse trial at 15 kg da^{-1} (NPK 15-15-15).

Identification of appropriate bacterial isolates

The plant materials and PGPR isolates used in the experiment are shown in Table 1. To select the PGPR isolates for the study, three isolates available in the stocks of Van Yuzuncu Yil University, Faculty of Agriculture, Department of Plant Protection, and whose efficacy had been demonstrated in previous studies, were used.

Table 1

Plant Materials and Bacterial Isolates Used in the Study

Plant materials		PGPR isolates	
Field trial	Greenhouse trial	Code	Species names
Great	Chianti	FZB42	*Bacillus amyloliquefaciens*
Kıvırcık	Defne	CC44	*Pseudomonas fluorescens*
Iceberg	Bombolo	CC37/2	*Pantoea agglomerans*

Growing medium characteristics

In the experiment, peat-perlite mixture was used at a ratio of 3:1 and 72-vials were used as seedling growing medium. [Peat content: EC: 35 mS/m, pH: 5.5-6.5, Fertilizer content: 1.0 kg/m^3; Perlite content: SiO_2 (72.0 - 76.0 %), Al_2O_3 (11.0 - 17.0 %), K_2O (4.0 - 5.0 %), Na_2O (2.9 - 4.0 %), CaO (0.5 - 2.0 %), MgO (0. 1 - 0.5 %), Fe_2O_3 (0.5 - 1.5 %), TiO_2 (0.03 - 0.2 %), MnO_2 (0.03 - 0.1 %), SO_3 (0 - 0.2 %), H_2O (2 - 7 %).]

Location of the Research Site

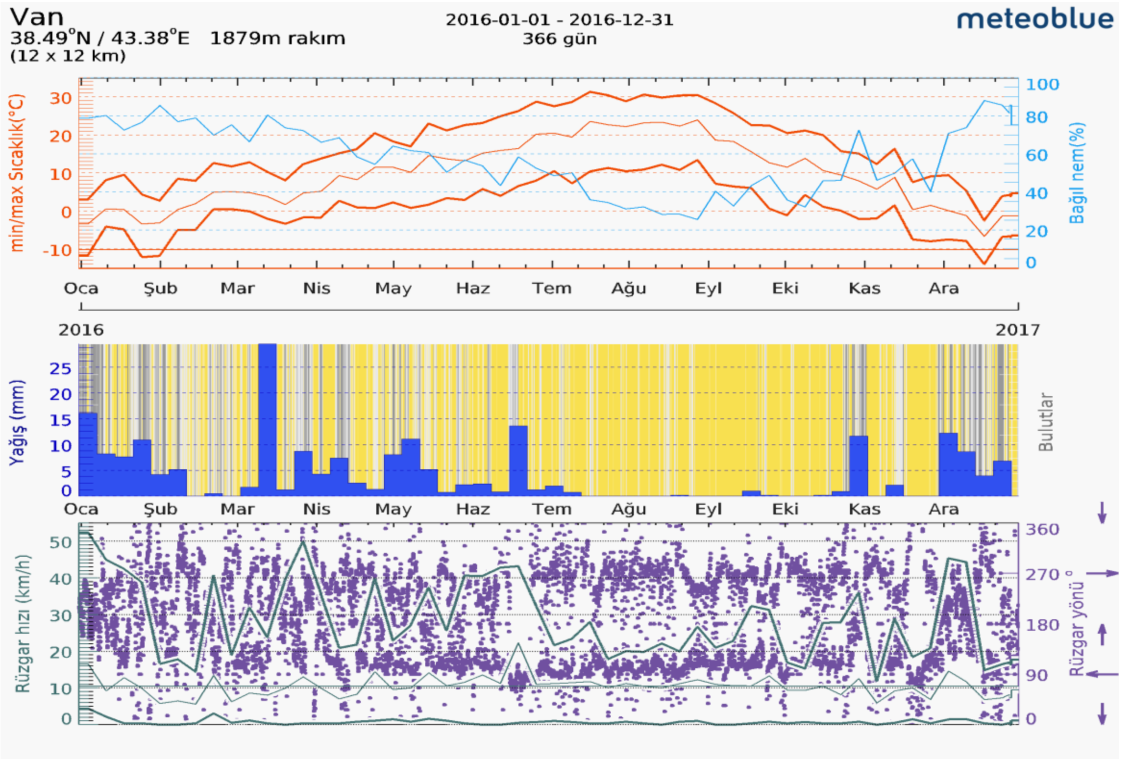
The research was conducted in the experimental field of Van Yuzuncu Yil University Research and Application Farm in 2016. The field trial was conducted between April 14 and August 12, and the greenhouse trial was conducted between October 2 and December 15. Van province is located in a basin surrounded by mountains to the west of Lake Van in the Eastern Anatolia Region, 1720 m above sea level and 38-25' north latitude and 43-21' east longitude. The trial area is located northeast of Lake Van, approximately 2 km from the lakeshore.

Climate Characteristics of the Research Site

Van has a continental climate with cold and snow-covered winters and cool and dry summers. Being located on the shores of Lake Van makes the climate of the province relatively mild. Monthly climate data for the periods of the study are presented in Figure 1. The rainfall during the growing season was 387.2 mm, the average temperature was 9.37 °C and the average relative humidity was 55.20%. In 2016, rainfall was 442.3 mm, average temperature was 9.85 °C and average relative humidity was 50.53% (Anonymous, 2018).

Figure 1

Some Climate Data for Van Province in 2016



Temperature was also measured inside and outside the greenhouse (°C), and the temperature inside the greenhouse varied ±8-13°C from the outdoor temperature.

Soil characteristics of the research site

Some physical and chemical analyses of the soil samples taken from 0-30 cm from the experimental area where the research was conducted were carried out in Van Commodity Exchange Laboratory and the results of the analysis are shown in Table 2.

Table 2

Field and Greenhouse Parcels' Soil Analysis Results

Field Soil Analyses	**Results**	**Status**
Potassium (K_2O)	131.7918	High
Phosphorus (P_2O_5)	6.6983	Medium
Lime (%)	7.3429	Moderately calcareous
Organic Matter (%)	0.5039	Very low
Total Salt (%)	0.0060	Salt-free
pH	7.16	Slightly alkaline
Saturation (%)	27	Sandy
Greenhouse Soil Analyses	**Results**	**Status**
Potassium (K_2O)	265.4233	High
Phosphorus (P_2O_5)	6.4258	Medium
Lime (%)	8.9274	Moderately calcareous
Organic Matter (%)	2.1639	Medium
Total Salt (%)	0.0045	Salt-free
pH	7.44	Neutral
Saturation (%)	33	Loamy

Methods

Plant cultivation

For the field trial, 4 varieties of head lettuce and 3 different PGPR isolates were planted in 72-well vials with 3 replicates (April 14, 2016). The field trial was conducted between April 14 and August 12, and the greenhouse trial was conducted between October 2 and December 15, determined as 110-120 days after sowing and 90-100 days after transplanting. The greenhouse trial determined this period as 70-80 days from seedling planting.

PGPR Applications

Each root bacterial isolate was grown in KB medium for 48 hours at 24°C. Bacterial cultures were suspended with 1.5% CMC. PGPR treatments were performed one week apart, starting at seedling emergence. At seedling emergence, a concentration of 10^9 cfu/ml was applied to the roots by inoculation.

Determination of Seedling Development Parameters

Determination of the number of head lettuce leaves: Determined by counting at the end of the experiment.

Head lettuce diameter and height measurements (cm): The head diameter and height were measured with a ruler.

Determination of head weight (g): Head lettuce weights were measured with a precision balance (±1g).

Determination of TSS content (°Brix): The total soluble solid content in the juice obtained from lettuce plants was measured using a hand refractometer.

Determination of leaf pH: The sap obtained by crushing the plant leaves in a ceramic mortar was measured with a pH meter.

Statistical analysis

The data obtained were analyzed using analysis of variance (one-way ANOVA) within the SPSS software package (IBM SPSS Statistics 21.0) according to the randomized block experimental design. The means were separated by "Duncan Multiple Comparison Test".

RESULTS AND DISCUSSION

Field Trial

Effect of PGPR Applications on Leaf Number

In the present study, the effects of PGPR on leaf number of different head lettuce (*Lactuca sativa* var. *capitata*) cultivars were investigated (Table 3). Significant differences ($P \leq 0.001$) were found among the cultivars. The cv. Kıvırcık gave the highest value with an average number of 25.88 leaves. It was followed by cv. Great (15.83 leaves) and Iceberg (15.21 leaves) varieties, respectively. In terms of PGPR treatments, the highest average number of leaves was found in CC37/2 isolate (20.55). No statistically significant difference was observed in the cultivar x PGPR interaction. Karagöz and Kotan (2010) reported that although PGPR isolates had positive effects on some parameters such as leaf number, these increases were not significant.

Table 3

Effects of PGPR Treatments on the Average Leaf Number of Different Head Lettuce Cultivars under Field Conditions

	CULTIVARS			
PGPR	**GREAT**	**KIVIRCIK**	**ICEBERG**	**MEAN**
CONTROL	14.17 ns	26.67	14.50	18.44 ns
CC37/2	17.33	**29.00**	15.33	20.55
CC44	**13.67**	27.17	14.50	18.44
FZB42	18.17	20.67	16.50	18.44
MEAN	15.83 B***	25.88 A	15.21 B	

***: Significant at $P \leq 0.001$ level

ns: not significant, there is no statistical difference between means.

Effect of PGPR Treatments on Head Diameter

The effects of PGPR treatments on head diameter in different head lettuce cultivars are presented in Table 4. Among PGPR isolates, CC37/2 isolate gave the highest value with 12.16 cm ($P \leq 0.05$). Iceberg variety had the highest head diameter with an average of 11.48 cm. No significant difference was found in the cultivar x PGPR interaction. Kesimci (2013) and Kıdoğlu *et al*. (2007) reported that the effects of PGPR and plant activators on head diameter were generally insignificant.

Table 4

Effects of PGPR Treatments on Average Head Diameter of Different Head Lettuce Cultivars under Field Conditions (cm)

	CULTIVARS			
PGPR	**GREAT**	**KIVIRCIK**	**ICEBERG**	**MEAN**
CONTROL	11.11 ns	**9.89**	10.15	10.38B*
CC37/2	12.69	10.21	**13.59**	12.16A
CC44	11.05	10.05	12.16	11.09AB
FZB42	11.64	10.10	10.00	10.58AB
MEAN	10.46 ns	10.06	11.48	

*: Significant at $P \leq 0.05$ level.

ns: not significant, there is no statistical difference between means.

Effect of PGPR Applications on Head Height

The effects of PGPR treatments on head height of head lettuce cultivars are given in Table 5. Among the varieties, cv. Kıvırcık had the highest head height with 14.00 cm ($P \leq 0.001$). Among PGPR isolates, CC37/2 isolate showed the highest value with 12.05 cm. No significant difference was found in the cultivar x PGPR interaction. Malkoçlu (2016) and Sadak *et al*. (2021) reported that the effect of PGPR applications on plant height was generally statistically insignificant in similar studies.

Table 5

Effects of PGPR Treatments on Average Head Height of Different Head Lettuce Cultivars under Field Conditions (cm)

	CULTIVARS			
PGPR	**GREAT**	**KIVIRCIK**	**ICEBERG**	**MEAN**
CONTROL	10.50 ns	13.50	10.00	11.33 ns
CC37/2	10.50	**14.83**	10.83	12.05
CC44	**9.67**	15.50	10.33	11.83
FZB42	10.33	12.17	11.00	11.17
MEAN	10.25 B***	14.00 A	10.54 B	

***: Significant at P≤0.001 level.

ns: not significant, there is no statistical difference between means.

Effect of PGPR Treatments on Head Weight

The effects of PGPR treatments on head weight of lettuce cultivars are shown in Table 6. Among PGPR isolates, CC37/2 isolate gave the highest result with 405.55 g (P≤0.05). The cv. Great had the highest head weight with 452.91 g (P≤0.001). No significant difference was found in the cultivar x PGPR interaction. Merdin (2009) and Malkoçlu *et al*. (2016) emphasized that the effects of PGPR on head weight were generally insignificant.

Table 6

Effects of PGPR Treatments on Average Head Weight of Different Head Lettuce Cultivars under Field Conditions (g)

	CULTIVARS			
PGPR	**GREAT**	**KIVIRCIK**	**ICEBERG**	**MEAN**
CONTROL	400.00 ns	**226.00**	299.33	308.44AB*
CC37/2	**570.00**	244.33	402.33	405.55A
CC44	342.33	265.00	284.68	297.33B
FZB42	499.33	232.00	257.00	329.44AB
MEAN	452.91 A***	241.83 B	310.83 B	

*: Significant at P≤0.05 level.

***: Significant at P≤0.001 level.

ns: not significant, there is no statistical difference between means.

Effect of PGPR Applications on Leaf pH

The effects of PGPR treatments on leaf pH are presented in Table 7. The cv. Great had the highest pH value with 6.49 (P≤0.001). FZB42 isolate gave the highest result with a pH value of 6.43 (P≤0.05). No significant difference was found in the cultivar x PGPR interaction. Kesimci (2013) reached similar results and reported that PGPR treatments had no significant effect on pH.

Table 7

Mean Leaf pH Values of PGPR Treatments in Different Head Lettuce Cultivars under Field Conditions

	CULTIVARS			
PGPR	**GREAT**	**KIVIRCIK**	**ICEBERG**	**MEAN**
CONTROL	6.43 ns	6.20	6.33	6.32 B*
CC37/2	6.53	6.17	6.47	6.39 AB
CC44	6.40	**6.13**	6.43	6.32 B
FZB42	**6.57**	6.17	**6.57**	6.43 A
MEAN	6.49 A***	6.17 B	6.45 A	

*: Significant at P≤0.05 level.

***: Significant at P≤0.001 level.

ns: not significant, there is no statistical difference between means.

Effect of PGPR Treatments on TSS content

The effects of PGPR treatments on the TSS content of head lettuce cultivars are given in Table 8. The cv. Kıvırcık had the highest TSS value with 5.00 °Brix. However, no significant difference was found between PGPR treatments. There was also no significant difference in the cultivar x PGPR interaction.

Table 8

The Effects of PGPR Treatments on the Average TSS Content of Different Head Lettuce Cultivars under Field Conditions (°Brix)

	CULTIVARS			
PGPR	**GREAT**	**KIVIRCIK**	**ICEBERG**	**MEAN**
CONTROL	4.33 ns	**5.00**	**5.00**	4.78 ns
CC37/2	4.67	4.33	4.33	4.44
CC44	4.00	**5.00**	4.00	4.33
FZB42	**3.33**	5.67	4.33	4.44
MEAN	4.08 ns	5.00	4.41	

ns: not significant, there is no statistical difference between means.

These results indicate that PGPR applications have various effects on plant growth, but these effects vary depending on the cultivar and isolates applied.

Protected cultivation (Greenhouse) trial

Effects of PGPR on the Number of Leaves in Different Head Lettuce Cultivars

PGPR treatments caused significant differences in the number of leaves in head lettuce cultivars (Table 9). Bombolo cultivar had the highest number of leaves with an average of 18.00 leaves, while cv. Chianti showed the lowest number of leaves. Significant differences in the number of leaves were also observed among PGPR isolates. Especially CC44 isolate formed significantly more leaves than the others. In the control treatment, the number of leaves remained lower. Similarly, Sadak *et al*. (2021) reported that the effect of PGPR on leaf number in pepper seedlings was insignificant, while Çiylez and Eşitken (2018) reported that some PGPR treatments were effective in strawberries. Our findings, as in these studies, show that PGPR has variety and isolate independent effects on plant growth.

Table 9

Effects of PGPR Treatments on The Average Number of Leaves of Different Head Lettuce Cultivars under Greenhouse Conditions

	CULTIVARS			
PGPR	**CHIANTI**	**DEFNE**	**BOMBOLO**	**MEAN**
CONTROL	**12.67** ns	15.83	**18.67**	15.72AB*
CC37/2	14.67	14.33	16.67	15.22B
CC44	15.17	18.17	**18.67**	17.33A
FZB42	13.17	16.00	18.00	15.72AB
MEAN	13.91 C***	16.09 B	18.00 A	

*: Significant at P≤0.05 level.

***: Significant at P≤0.001 level.

ns: not significant, there is no statistical difference between means.

Effects of PGPR on Head Diameter in Different Head Lettuce Varieties

In head diameter measurements, cv. Bombolo had the largest head diameter, while no significant difference was observed between the other cultivars (Table 10). Among PGPR treatments, CC44 isolate provided the largest head diameter. Studies such as Sadak *et al*. (2021) and Ekici *et al*. (2015) indicated that PGPR treatments can positively affect head diameter. However, in this study, the effect of PGPR on head diameter did not differ among varieties.

Table 10

Effects of PGPR Treatments on Average Head Diameter of Different Head Lettuce Cultivars Under Greenhouse Conditions (cm)

	CULTIVARS			
PGPR	**CHIANTI**	**DEFNE**	**BOMBOLO**	**MEAN**
CONTROL	9.73 ns	**9.46**	10.89	10.03 ns
CC37/2	9.68	9.57	10.21	9.82
CC44	10.26	11.16	10.95	10.79
FZB42	9.52	10.10	**11.59**	10.40
MEAN	9.80 ns	10.07	10.91	

ns: not significant, there is no statistical difference between means.

Effects of PGPR on Head Height in Different Head Lettuce Varieties

In the head height results, cv. Bombolo reached the highest plant height, while the head height of the control group was the highest among PGPR treatments (Table 11). Kesimci (2013) and other studies reported that the effects of bacterial treatments on head height were generally limited, and our findings showed similar results.

Table 11

Mean Head Height (cm) of Different Lettuce Cultivars of PGPR Treatments under Greenhouse Conditions

	CULTIVARS			
PGPR	**CHIANTI**	**DEFNE**	**BOMBOLO**	**MEAN**
CONTROL	13.83 ns	14.33	**14.50**	14.22 ns
CC37/2	13.50	**10.33**	13.17	12.33
CC44	11.50	15.33	14.00	13.61
FZB42	13.33	11.17	13.83	12.78
MEAN	13.04 ns	12.80	13.88	

ns: not significant, there is no statistical difference between means

Effects of PGPR on Head Weight in Different Head Lettuce Varieties

In head weight measurements, cv. Defne had the highest average head weight, while CC44 isolate provided the highest head weight among PGPR isolates (Table 12). Similarly, Moustaine *et al.* (2017) and Güneş (2018) reported that PGPR applications can positively affect head weight. Our findings show that some PGPR isolates can increase head weight in accordance with this literature.

Table 12

Effects of PGPR Treatments on Average Head Weight of Different Head Lettuce Cultivars under Greenhouse Conditions (g)

	CULTIVARS			
PGPR	**CHIANTI**	**DEFNE**	**BOMBOLO**	**MEAN**
CONTROL	89.00 ns	103.33	137.33	109.89
CC37/2	94.00	106.67	112.00	104.22
CC44	110.33	139.00	126.33	125.22
FZB42	**91.33**	106.33	**142.00**	113.22
MEAN	96.17 B*	113.83 AB	129.41 A	

*: Significant at P≤0.05 level.

ns: not significant, there is no statistical difference between means.

Effects of PGPR on pH in Different Head Lettuce Varieties

Among the PGPR isolates, CC44 isolate provided the highest pH (P≤0.05); the highest pH among the varieties was measured in cv. Chianti (Table 13). Öztekin *et al.* (2015) obtained similar results in pH values in tomato, and our findings showed that the differences in pH varied depending on specific isolates.

Table 13

Mean Leaf pH Values of PGPR Treatments in Different Head Lettuce Cultivars under Greenhouse Conditions

	CULTIVARS			
PGPR	**CHIANTI**	**DEFNE**	**BOMBOLO**	**MEAN**
CONTROL	6.47 ns	6.40	**6.27**	6.38 AB*
CC37/2	**6.27**	6.33	6.37	6.32 B
CC44	**6.60**	6.43	6.40	6.48 A
FZB42	6.40	6.37	6.40	6.39 AB
MEAN	6.43 ns	6.39	6.35	

*: Significant at P≤0.05 level.

ns: not significant, there is no statistical difference between means.

Effects of PGPR on TSS in Different Head Lettuce Cultivars

Chianti cultivar obtained the highest average TSS values, while FZB42 isolate provided the highest average TSS in PGPR treatments (Table 14). Studies such as Kesimci (2013) and Gök and Onaç (1995) have reported that the effects of PGPR on TSS may vary depending on cultivar and conditions. Our findings also support these variations.

Table 14

The Effects of PGPR Treatments on the Average TSS of Different Head Lettuce Cultivars under Greenhouse Conditions (°Brix)

	CULTIVARS			
PGPR	**CHIANTI**	**DEFNE**	**BOMBOLO**	**MEAN**
CONTROL	**6.93**ns	**5.13**	5.47	5.84 ns
CC37/2	6.40	6.27	6.43	6.37
CC44	6.07	6.07	6.70	6.28
FZB42	6.67	6.53	6.00	6.40
MEAN	6.51 ns	6.00	6.15	

ns: not significant, there is no statistical difference between means

Shao *et al*. (2023) studied different strains of PGPRs on lettuce and highlighted the role of specific bacterial strains in promoting lettuce growth and yield. The strains used in their study, such as *Bacillus velezensis* and *Bacillus amyloliquefaciens*, showed improvements in head diameter, plant height, and fresh weight, aligning with our findings on PGPR effects. Shao *et al*. (2023) also emphasized how lettuce variety impacts the effectiveness of microbial applications; discussed how benefits observed in pot trials do not always translate to field conditions; and stating that there was a lack of PGPR effectiveness across different environments.

Demir *et al*. (2023) studied biofertilizer (BM-MegaFlu®) comprised *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Pantoea agglomerans* bacteria on lettuce and brokoli, and they found when combining these bacteria with reduced doses of chemical fertilizers aligns with our findings about the benefits of specific PGPR strains like *Pseudomonas fluorescens* (CC44) and *Pantoea agglomerans* (CC37/2) on lettuce growth and yield. Their study emphasizes how combining biofertilizers with lower doses of chemical fertilizers can achieve comparable or even superior yields to full chemical treatments.

CONCLUSION

This study investigated the effects of different PGPR isolates on head lettuce under both field and greenhouse conditions. The results demonstrated that the impact of PGPR treatments on lettuce growth and yield varied significantly depending on plant variety, isolate type, and environmental factors such as climate and growing conditions. The findings indicate that specific bacterial strains can effectively promote lettuce growth, but their effectiveness may vary across different environments and cultivation methods.

In our study, the Kıvırcık variety yielded the highest number of leaves under field conditions, while the Bombolo variety excelled in greenhouse conditions. Although PGPR isolates had limited effects on leaf number, their impact on head diameter was notable, particularly in the field. The Iceberg variety exhibited the largest head diameter under field conditions, and Bombolo performed best in the greenhouse. Regarding head weight, the Great variety led in field trials, while the Defne variety excelled in greenhouse conditions. PGPR isolates significantly influenced head weight in field conditions, though their effect was more limited in greenhouse trials. In terms of pH, the Great and Chianti varieties recorded the highest leaf pH under field and greenhouse conditions, respectively, with PGPR isolates having a statistically significant impact across both environments.

The study suggests that selecting compatible PGPR strains for specific lettuce varieties is key to optimizing growth. Additionally, environmental factors in greenhouse conditions, like soil and moisture, should be carefully managed to enhance PGPR effectiveness. Combining PGPR isolates with reduced chemical fertilizers could be an effective strategy for sustainable lettuce production. Future research should focus on conducting broader field trials across diverse regions to validate and refine these approaches.

In conclusion, while this study confirms the potential of PGPR isolates to enhance lettuce yield and quality, the outcomes are highly dependent on plant variety, environmental conditions, and isolate type. Continued research is needed to refine these treatments and fully integrate them into sustainable agricultural practices.

Ethical Statement

The article is adapted from the first author's M.Sc. thesis. The authors would like to thank Dr. A. Akkopru, Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Plant Protection, for the PGPR supply.

Author Contributions

Research Design (CRediT 1) Author 1 (%50) – Author 2 (%50)

Data Collection (CRediT 2) Author 1 (%70) – Author 2 (%30)

Research - Data Analysis - Validation (CRediT 3-4-6-11) Author 1 (%50) – Author 2 (%50)

Writing the Article (CRediT 12-13) Author 1 (%60) – Author 2 (%40)

Revision and Improvement of the Text (CRediT 14) Author 1 (%20) – Author 2 (%80)

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12 Responsible Production and Consumption

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Assessment of the Effect of Plant Growth Promoting Rhizobacteria (PGPR) Strains on Rooting in Rose Cuttings

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ABSTRACT

Roses (*Rosa* spp.), members of the *Rosaceae* family, are widely cultivated ornamental plants with significant commercial value worldwide. Vegetative propagation of roses is typically achieved through cuttings; however, this process often results in substantial losses. This study investigated the effects of plant growth-promoting rhizobacteria (PGPR) applications on the rooting performance of garden rose cuttings. The PGPR strains used in the study were identified via PCR analyses for the presence of the indole acetic acid (IAA) gene region, and three different formulations were prepared: Formulation 1 contained only *Bacillus subtilis*, Formulation 2 contained only *Bacillus cereus*, and Formulation 3 was a mixture of *Bacillus subtilis* and *Bacillus cereus*. The results demonstrated that PGPR applications significantly enhanced the rooting rate of rose cuttings. Compared to the control group, the PGPR-treated cuttings exhibited a 50-60% increase in rooting rate, supporting seedling root development. These findings indicated that Bacillus species rhizobacteria had positive effects on the rooting process in rose cuttings. The study emphasizes the potential of using environmentally friendly biostimulants, such as PGPR, in the ornamental plant industry as an alternative to hormones and chemicals. In conclusion, it is recommended that PGPR-based biostimulants be considered as a sustainable and effective alternative for rose cultivation.

Bitki Gelişimini Teşvik Eden Rizobakteri (PGPR) Suşlarının Gül Çeliklerinde Köklenme Üzerine Etkisinin Değerlendirilmesi

Makale Bilgisi

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Anahtar Kelimeler:
PGPR,
Bacillus,
Süs bitkisi,
Gül,
IAA.

ÖZET

Gül (*Rosa* spp.), *Rosaceae* familyasının bir üyesi olarak dünya genelinde yaygın şekilde yetiştirilen ve önemli bir ticari değere sahip olan süs bitkilerindendir. Güllerin vegetatif olarak çoğaltılması genellikle çelikler aracılığıyla yapılmakta olup, bu süreçte önemli oranda kayıplar yaşanabilmektedir. Bu çalışmada, bitki büyümesini teşvik edici rizobakteri (PGPR) uygulamalarının bahçe tipi gül çeliklerinin köklenme performansı üzerindeki etkileri araştırılmıştır. Çalışmada kullanılan PGPR suşları, PCR analizleri ile indol asetik asit (IAA) gen bölgesi açısından tespit edilmiş ve bu suşlardan üç farklı formülasyon hazırlanmıştır: Formülasyon 1 yalnızca *Bacillus subtilis*, Formülasyon 2 yalnızca *Bacillus cereus* ve Formülasyon 3 *Bacillus subtilis* ile *Bacillus cereus* karışımını içermektedir. Elde edilen sonuçlar, rizobakteri uygulamalarının gül çeliklerinin köklenme oranını anlamlı şekilde artırdığını ortaya koymuştur. PGPR uygulamaları, kontrol grubuna kıyasla yeşil çeliklerin köklenme oranında %50-60 oranında bir artış sağlamış ve fide kök gelişimini desteklemiştir. Bu bulgular, özellikle Bacillus türü rizobakterilerin gül çeliklerinde köklenme sürecine pozitif katkıda bulunduğunu göstermektedir. Çalışma, hormonlar ve kimyasal maddeler yerine çevre dostu biyostimülantlar olarak PGPR uygulamalarının süs bitkileri sektöründe geniş kullanım potansiyeline sahip olduğunu vurgulamaktadır. Sonuç olarak, PGPR gibi biyostimülantların gül yetiştiriciliğinde sürdürülebilir ve etkili bir alternatif olarak değerlendirilmesi gerektiği önerilmektedir.

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INTRODUCTION

The rose (*Rosa* L.), belonging to the genus *Rosa* in the family Rosaceae, is a valuable ornamental plant used both in landscape design and as a cut flower. It ranks first among globally cultivated ornamental plants due to its flowers, essential oil, and other value-added products. Modern roses are predominantly hybrids derived from diploid and tetraploid species native to Asia and Europe (Zhang et al. 2013).

Research and development of new rose varieties continue worldwide. The vegetative propagation of varieties bred in accordance with breeding objectives is generally carried out through the method of cutting propagation. The cutting propagation method is extensively preferred in the vegetative propagation of ornamental plants due to the identical genetic structure between the parent and the newly produced plant. However, several factors influence the process of cutting production. Among these factors are the age and characteristics of the parent plant from which the cutting is taken, the season of cutting collection, the type of cutting, the storage conditions until planting, chemical substances used to accelerate rooting, the physical and chemical properties of the rooting medium, the temperature of the rooting medium, sterilization substances used to prevent fungal infections (Dalda-Sekerci and Ünlü, 2023).To hasten root formation and promote healthy root development in plants propagated via cuttings, various chemical substances and hormonal treatments are frequently utilized. However, in recent years, the importance of microorganisms has risen within the context of sustainable agricultural practices. Research is increasingly focusing on the use of these microorganisms for biocontrol of plant diseases, enhancement of plant growth, bio-fertilization, and improvement of rooting efficiency in cuttings (Antoun and Prevost, 2006; Mehmood et al., 2018; Ünlü et al., 2023). Studies conducted thus far have underscored the primary role of bacteria among the key beneficial microorganisms utilized in plant production (Higa and Paar, 1994; Bloemberg and Lugtenberg, 2001; Esitken et al., 2003; Lugtenberg and Kamilova, 2009).

These studies have revealed that the application of rhizobacteria species, including *Bacillus, Pseudomonas, Agrobacterium, Streptomyces*, and *Alcaligenes*, as well as the use of Indole Acetic Acid (IAA)-producing bacteria with genes responsible for IAA production, significantly promotes rooting in cuttings (Esitken et al., 2003; Kisvarga et al., 2022). Studies have demonstrated that Plant Growth-Promoting Rhizobacteria (PGPRs) are utilized as plant growth regulators in ornamental plant cultivation worldwide due to their capacity to enhance plant growth, yield, and soil quality (Srivastava and Govil, 2007; Sharma and Kaur, 2010; García-Fraile et al., 2012; Flores-Félix et al., 2013; Zulueta-Rodriguez et al., 2014; Karagöz et al., 2016). Typically, these bacteria colonize the root system, supporting plant development and suppressing harmful microorganisms. PGPRs facilitate plant growth by producing growth hormones, regulating microbial balance in the rhizosphere, and enhancing mineral uptake, thereby positively influencing plant development (Siddiqui, 2006; Şevik, 2010). Additionally, growth-promoting rhizobacteria stimulate rooting and enable the production of high-quality seedlings in a shorter time frame (Ruzzi and Aroca, 2015). Rhizobacteria not only promote rooting but also contribute to healthier seedlings through faster and superior root formation (Şekerci and Ünlü, 2023).

In this study, three distinct rhizobacterial formulations (*Bacillus subtilis*, *Bacillus cereus*, and mix of *Bacillus subtilis* and *Bacillus cereus*) were applied to assess their impact on the rooting of green rose cuttings.

MATERIAL AND METHODS

Plant Material

Semi-hardwood cuttings, each containing 3-4 buds, obtained from pink garden roses, were employed as the plant material.

Rhizobacteria Isolation from Soil

Sixty soil samples were collected from various locations across Central Anatolia, Turkey, for the isolation of rhizobacteria. These samples were transferred into bottles containing sterile water (0.9% NaCl) and Luria-Bertani (LB) Broth medium. After incubation, aliquots from each sample were spread onto solid LB and NB (Nutrient Broth) media using the spread plate technique. The plated samples were then incubated at 35°C for 12-24 hours to facilitate the growth of primary bacterial cultures. Then, isolates from distinct colonies on the petri dishes were selected and purified. Purified strains were further cultured and transferred to NB medium (Upadhyay et al., 2009).

DNA Isolation from Rhizobacteria

The bacterial DNA was extracted according to the procedure of Wilson (2001). Bacterial isolates were cultured in 10 mL of nutrient broth (NB) and incubated for 24 hours. After incubation, 1.5 mL of the bacterial culture was suspended in TE (Tris-EDTA) buffer (pH 8.0). To each suspended pellet, 30 µL of 10% SDS (sodium dodecyl sulfate) and 3 µL of proteinase K were added, mixed thoroughly, and then incubated at 37°C for 1 hour. Following the incubation, 100 µL of 5M NaCl and 80 µL of CTAB/NaCl solution were added to each sample. The samples were then washed sequentially with chloroform/isoamyl alcohol (24/1, v/v), phenol/chloroform/isoamyl alcohol (25/24/1, v/v/v), and isopropanol, and finally dissolved in TE (Tris-EDTA) buffer.

PCR Amplification and Sequencing of the 16S rDNA Gene

The DNA obtained from the bacteria was initially tested with IAA primers F 5'-CCAACATCATCAAGCTGCCGAACA-3' and R 5'-AGACCTTCATCATCGTGGCCTTCA-3', and bacterial strains possessing the IAA gene region were identified. The identification of the isolates based on 16S rDNA sequence analysis involved the following steps: isolation of genomic DNA, amplification using universal 16S forward (5'-3') and 16S reverse (3'-5') primers (targeting 16S rDNA regions; 16S forward 5'-AGA GTT TGA TCC TGG CTC AG-3' and 16S reverse 5'-CCG TCA ATT CCT TTG AGT TT-3') according to Edwards et al. (1989), sequencing of the amplified regions, and comparison of the obtained sequences with the base sequences of microorganisms available in the database.

Preparation and Activation of Rhizobacteria

Different rhizobacteria solutions were prepared from bacteria identified as carrying the IAA (indole acetic acid) gene through PCR analysis. These rhizobacteria were reactivated from stock cultures stored at -80°C, resulting in three distinct formulations (Table 1). Nutrient Agar (NA) and Nutrient Broth (NB) media were employed for the reactivation and cultivation of the rhizobacteria (Yılmaz, 2010; Ünlü et al., 2023). Bacterial suspensions were prepared at a concentration of $3x10^7$ cfu/ml.

Table 1

Formulations created by activating stock bacterial strains used in the study

Treatments	Bacteria Code	Bacteria Name	rate
1	61.29 e*	*Bacillus subtilis*	1/1
2	33B-EÜ*	*Bacillus cereus*	1/1
3	61.29 e* 33B-EÜ*	*Bacillus subtilis* *Bacillus cereus*	½ ½
Control	-	Medium	-

* Defined by MAlDI TOF

Application of Bacteria to Cuttings

Semi-hardwood cuttings of pink garden roses, prepared with 3-4 buds, were soaked in rhizobacteria solution for 5 minutes before planting. In the control group, the cuttings were soaked in water. The rose cuttings were planted in growth containers filled with a 1:1 mixture of peat and perlite, with each container holding 15 cuttings and replicated three times. After planting, the cuttings were watered twice with 5 ml ($3x10^7$ cfu/ml) of bacterial solution mixed into 1 liter of irrigation water at 15-day intervals.

Statistical Analyses

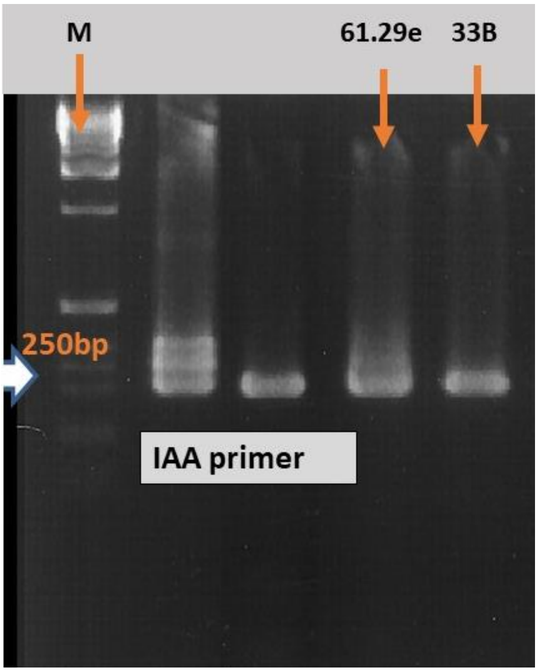
Rooting rate measurements were recorded at the end of the 10th week to assess the effects of different bacterial formulation treatments. The obtained data were analyzed using analysis of variance (ANOVA) in SAS software (version 9.00). Means were compared using the Duncan test at significance levels of 0.05.

RESULTS AND DISCUSSION

Propagation via cuttings is a widely used method in rose cultivation. However, this method often results in seedling losses due to the proliferation of fungal and bacterial diseases. In this study, three different rhizobacterial formulations were tested to determine their effects on the rooting of semi-hardwood cuttings of pink garden roses. PGPR bacteria, isolated from various soils, had their DNA extracted and subjected to PCR analysis using IAA primers. Subsequently, DNA fragments containing the target 16S rDNA gene were extracted. The analysis revealed that the rhizobacterial species used in the study possessed the IAA gene region (Figure 1).

Figure 1

PCR gel images of Bacillus subtilis and Bacillus cereus species indole acetic acid (IAA) gene region



Rhizobacterial treatments provided a statistically significant increase in rooting compared to the control group (Table 2, Figure 2). The rooting success rates were as follows; control group 40.75%, *B. cereus* treatment 62.50%, *B. subtilis* treatment 50.25%, and mixed application of *B. cereus* and *B. subtilis* strains 74.75%. These results indicate that *Bacillus* spp. positively affect rooting in rose cuttings (Figure 3).

Table 2

The effect of rhizobacteria treatments on cutting rooting rate in rosa

Treatments	Rhizobacteria	Rooting rate (%)
Formulation 1	*Bacillus subtilis,*	50,25c
Formulation 2	*Bacillus cereus*	62,50b
Formulation 3	*Bacillus subtilis 1/2* *Bacillus cereus 1/2*	74,75a
Control		40,75c
CV		**1,215**
LSD		**11,88**

Figure 2

Visual of the effect of 1st formulation (B. subtilis), 2nd formulation (B. cereus), and 3rd formulation (B. subtilis and B. cereus) and control on rooting in rosa.

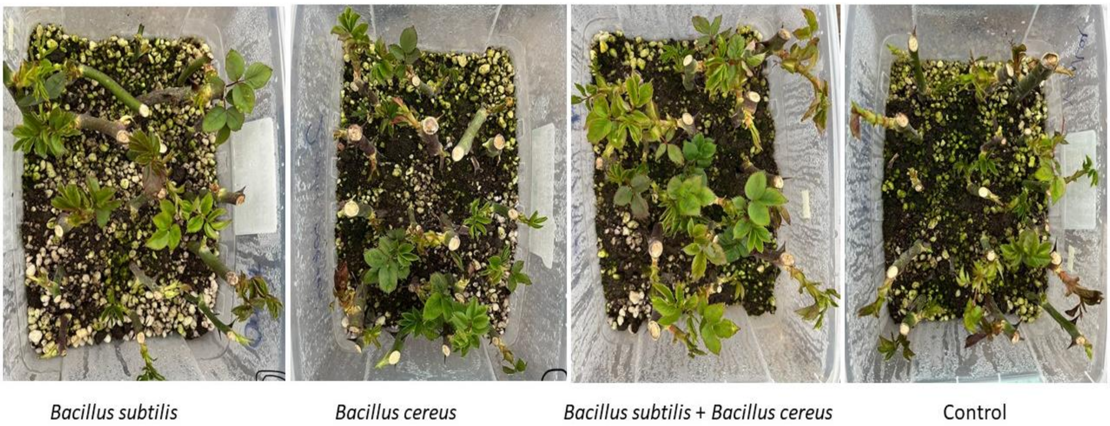
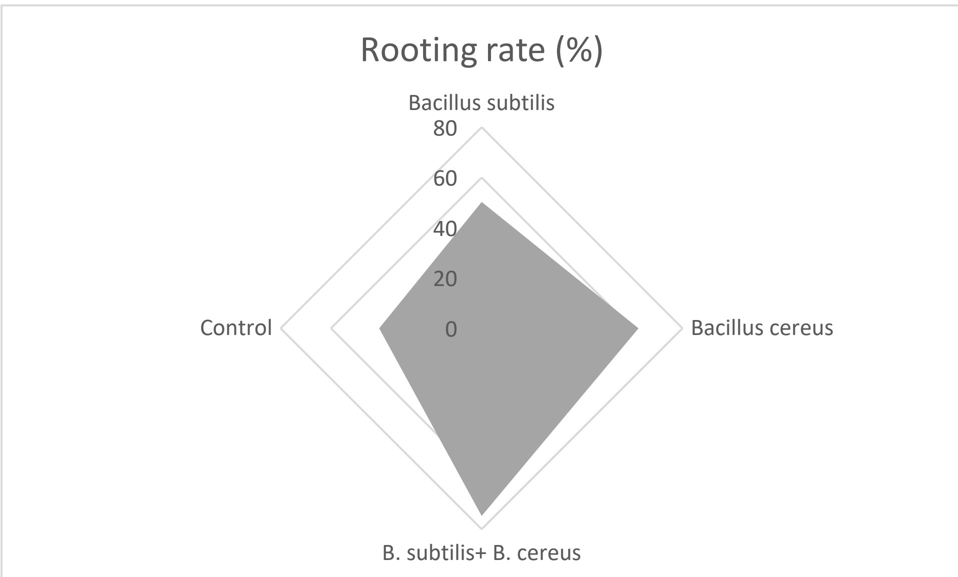


Figure 3

Graphical Representation of the Impact of Various PGPR Bacterial Strains on Rose Rooting Parameters



The present study clearly demonstrates the positive effects of different *Bacillus* species on the rooting of rose cuttings. In recent years, there has been a noticeable increase in the use of rhizobacterial applications in ornamental plant cultivation, a trend that extends throughout the horticultural sector. Despite advancements in vegetative propagation techniques, the industry still faces economic challenges due to suboptimal rooting efficiency. Previous studies have highlighted the need for further research to identify biostimulants that can enhance root development (Ahkami et al., 2009). The findings of this study align with the literature, providing consistent data with past research. Numerous studies have confirmed the effectiveness of rhizobacterial applications in promoting root growth and natural plant development, suggesting a promising alternative to commercial hormones.

Positive outcomes from the application of plant growth-promoting rhizobacteria (PGPR) have been documented in various commercially important ornamental plants, showing beneficial effects on both rooting processes and agronomic characteristics. Research has explored the use of PGPR bacteria in ornamental plants belonging to various botanical families, including *Asteraceae* (e.g., chrysanthemum, aster, and zinnia) and *Geraniaceae* (e.g., geranium) (Göre and Altın, 2006), *Iridaceae* (iris) and *Oleaceae* (jasmine) (Damodaran et al., 2014), *Solanaceae* (petunia) (Hoda and Mona, 2014), *Crassulaceae* (kalanchoe) (Dalda-Sekerci and Ünlü, 2023) and *Poaceae* (turfgrass) (Okumus et al., 2024). The results of these studies are consistent with previous research, emphasizing the positive impact of PGPR applications on rooting across a variety of ornamental plants. For instance, earlier studies showed that certain strains of *Pseudomonas fluorescens* enhanced rooting in zinnia flowers (Yuen and Schroth, 1986). In firethorn cultivation, experiments combined indole-3-butyric acid with *Azospirillum brasilense* strains to promote early shoot rooting, encouraging rooting at an early developmental stage (Larraburu et al., 2007). Similarly, another study revealed that the presence of *Agrobacterium rubi* and *Serratia liquefaciens* significantly increased both fresh and dry root weight during the rooting process of hardwood cuttings derived from Forsythia intermedia plants (Kır, 2010). Sezen et al. (2014) examined the effects of *Agrobacterium rubi*, *Pseudomonas putida,* and *Bacillus subtilis* on the rooting process of *Ficus benjamina* L. cuttings and found that Bacillus subtilis exhibited the highest efficacy compared to the other bacterial species.

Similarly, Alkaç et al. (2022) investigated bacterial applications on aster flowers and observed varied effects; notably, the application of *Pseudomonas putida* (ZE-12) resulted in a 12% increase in germination compared to the control group, while *Acinetobacter calcoaceticus* (ZE-13) led to a significant 32.9% increase in seedling height. It is well-documented that *Bacillus* spp. promote plant growth by synthesizing plant growth regulators such as indole-3-acetic acid (IAA), gibberellins, and cytokinins. The biosynthesis of IAA in bacteria is crucial in regulating various aspects of plant growth and development, including differentiation of root vascular tissue, lateral root formation, and root gravitropism, thus playing a fundamental role in shaping plant root structure (Aloni et al., 2006). Furthermore, a study on *Rosa canina* reported the highest rooting rate with the application of *Bacillus megaterium* and *Pseudomonas fluorescens* bacteria (Kınık, 2014). In summary, the use of PGPR bacteria in agriculture is of great significance and continues to be a focus of research due to its potential benefits.

CONCLUSIONS

In recent times, there has been a concerted effort among researchers to devise an integrated strategy aimed at mitigating the adverse impacts of synthetic chemicals utilized in agricultural practices. Within this framework, biostimulants have emerged as pivotal contributors, particularly within the realms of horticulture and ornamental plant cultivation. Additionally, biostimulants are progressively gaining traction for their role in bolstering tolerance to both biotic and abiotic stresses, as well as in enhancing sexual and asexual reproduction, seedling development, and overall yield. Among the noteworthy applications of biostimulants, treatments involving Plant Growth-Promoting Rhizobacteria

(PGPR) offer manifold benefits, including conservation of soil and water resources, mitigation of environmental pollution arising from pesticides and chemical fertilizers, management of diseases and pests, augmentation of nutrient uptake by plants, and alleviation of biotic and abiotic stresses in plants. Recent studies have yielded significant findings demonstrating the capacity of PGPRs to stimulate root formation, increase plant height, expand leaf area, enhance shoot and root dry weights, modulate flowering time, augment flower and branch numbers, and extend flowering durations in ornamental plants.

This investigation, specifically, revealed that formulations containing bacterial strains from the *Bacillus* genus notably enhanced rooting in rose cuttings. Hence, it is anticipated that future research endeavors will further refine the efficacy of biostimulants and widen their adoption in commercial settings, thereby unveiling their substantial potential within the agricultural sector.

Ethics Statement

This study was produced from the TUBITAK 2209-A student project submitted by Kanykei KANATBEKOVA under the supervision of Dr. Akife DALDA ŞEKERCİ.

Author Contributions

Research Design Author 1 (%100)

Data Collection Author 1 (%40) Author 2 (%30) Author 3(%30)

Research- Data Analysis Author 1 (%20) Author 2(%40) Author 3(%40)

Writing the article Author 1 (%80) Author 3(%20)

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Conflict of Interest

No conflict of interest.

Sustainable Development Goals (SDG)

12 Responsible Production and Consumption

13 Climate Action

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Effects of PGPR on Yield and Quality in Different Melon (*Cucumis melo* L.) Cultivars

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ABSTRACT

This study aimed to assess the impact of PGPR (Plant Growth-Promoting Rhizobacteria) on the yield and quality of various melon (*Cucumis melo* L.) cultivars under Van ecological conditions during the 2016/2017 season. The experiment utilized PGPR applications including FZB42 (*Bacillus amyloliquefaciens*), CC378/2 (*Pantoea agglomerans*), and CC44 (*Pseudomonas fluorescens*), with six melon cultivars: Kırkağaç 637, BT Akhisar, Napolyon F1, Lokma F1, Lokum F1, and Ananas. The study was conducted using a randomized block design with three replications. Results indicated that PGPR applications enhanced several growth and yield parameters: stem thickness increased by up to 9.2%, leaf length by up to 12.9%, petiole length by up to 8.3%, fresh leaf weight by up to 12.8%, dry leaf weight by up to 12.9%, average fruit yield per plant by up to 39.1%, average fruit weight by up to 21.9%, fruit flesh thickness by up to 17.6%, fruit width by up to 7.4%, fruit length by up to 9.2%, average number of branches by up to 21.1%, and total branch length by up to 13.2%.

Farklı Kavun (*Cucumis melo* L.) Çeşitlerinde PGPR Kullanımının Verim ve Kalite Üzerine Etkileri

Makale Bilgisi

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Anahtar Kelimeler:
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Kavun,
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Kalite
Verim

ÖZET

Bu araştırma, 2016/2017 sezonunda Van ekolojik şartlarında farklı kavun (*Cucumis melo* L.) çeşitlerinde PGPR (Bitki Gelişimini Destekleyen Rizobakteri) kullanımının verim ve kalite üzerindeki etkilerini belirlemek amacıyla yapılmıştır. Denemede, FZB42 (*Bacillus amyloliquefaciens*), CC378/2 (*Pantoea agglomerans*) ve CC44 (*Pseudomonas fluorescens*) izolatlarından oluşan PGPR uygulamaları kullanılmıştır. Denemede Kırkağaç 637, BT Akhisar, Napolyon F1, Lokma F1, Lokum F1 ve Ananas kavun çeşitleri kullanılmıştır. Araştırma, tesadüf blokları deneme desenine göre ve üç tekrar ile yürütülmüştür. Sonuçlar, PGPR uygulamalarının kavun bitkisinde; ana gövde kalınlığını %9.2, yaprak uzunluğunu %12.9, yaprak sapı uzunluğunu %8.3, yaş yaprak ağırlığını %12.8, kuru yaprak ağırlığını %12.9, bitki başına ortalama meyve verimini %39.1, ortalama meyve ağırlığını %21.9, meyve eti kalınlığını %17.6, meyve genişliğini %7.4, meyve boyunu %9.2, ortalama dal sayısını %21.1 ve toplam dal uzunluğunu %13.2 oranlarında artırdığını göstermiştir.

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INTRODUCTION

Melons (*Cucumis melo* L.) are a significant crop globally, valued for their sweet flavor, nutritional benefits, and economic importance. Melon is one of the most important vegetables species produced and consumed in almost every corner of Türkiye; as well as around the World (Erdinc *et al*., 2013, 2021). In the diverse ecological conditions of Van, the cultivation of melons presents unique challenges and opportunities. To enhance melon production, researchers and farmers are increasingly exploring sustainable agricultural practices, including the use of Plant Growth-Promoting Rhizobacteria (PGPR). PGPR are beneficial microorganisms that can improve plant growth, yield, and resilience by enhancing nutrient uptake, stimulating plant growth hormones, and suppressing plant pathogens.

In recent years, sustainable agricultural practices have become essential due to increasing environmental challenges, such as soil degradation, water scarcity, and climate change impacts on crop yields. PGPR has emerged as a promising solution to support plant resilience under such stresses, making it particularly valuable in areas with variable ecological conditions. PGPR not only enhances crop growth under optimal conditions but also improves plant tolerance to biotic or abiotic stresses by boosting antioxidant activities and aiding in osmotic balance (Bilge *et al*., 2019; Tunçtürk *et al*., 2019; Sadak *et al*., 2021). Given the susceptibility of melon crops to such stresses, particularly in regions like Van with diverse climates, understanding the role of PGPR can provide crucial insights for developing resilient and sustainable production systems. By integrating these beneficial bacteria into melon cultivation, growers may achieve consistent crop quality and yield even under suboptimal conditions

Previous research has highlighted the effectiveness of PGPR in various horticultural crops. PGPRs directly affect plant growth by producing growth hormones and altering the microbial balance in the rhizosphere. They also protect the plant against diseases by suppressing soil-borne pathogens (Siddiqui, 2006; Bilge *et al*., 2019; Tunçtürk *et al*., 2019). For example, Demir *et al*. (2023) demonstrated that biofertilizers, including PGPR like *Bacillus megaterium* and *Pseudomonas fluorescens*, significantly improved plant growth, yield, and nutrient concentration in lettuce and broccoli under greenhouse conditions. Similarly, studies by Zapata-Sifuentes *et al*. (2022) showed that PGPR can enhance growth parameters and fruit quality of cucumber under greenhouse conditions. These findings highlight the potential of PGPR to positively influence plant development and productivity.

Despite these promising results, research specifically targeting the effects of PGPR on melon cultivars, especially in the distinct ecological conditions of Van, is limited. This study aims to address this gap by evaluating the impact of four different PGPR isolates—Control, *Bacillus amyloliquefaciens* (FZB42), *Pantoea agglomerans* (CC378/2), and *Pseudomonas fluorescens* (CC44)—on six melon cultivars (Kırkağaç 637, BT Akhisar, Napoleon F1, Lokma F1, Lokum F1, and Ananas). By examining growth parameters such as stem thickness, leaf dimensions, fruit characteristics, and branch development, this research seeks to provide valuable insights into the benefits of PGPR application for optimizing melon production in Van's ecological conditions. The results are anticipated to contribute to more effective and sustainable melon cultivation practices, enhancing both yield and fruit quality in the region.

MATERIALS AND METHODS

Plant Materials

The study used the following melon cultivars:

Kırkağaç 637: Early maturing, strong plants with round-oval fruits (2.5-3 kg). Dark yellow skin with green spots, thick white flesh, and high sweetness. Long shelf life.

BT Akhisar Topan: Developed from Akhisar-Kırkağaç melons, round fruits (2-3 kg) with yellow skin and green spots. Sweet, small seed cavity, high yield, matures in 90-100 days.

*Napolyon F1***:** High yielding with good disease resistance. Homogenous fruits with high sugar content, suitable for open fields.

Lokma F1: Early maturing, high-yielding, and suitable for stringing. Round Galia-type fruits (2-2.5 kg) with good shelf life.

Lokum F1: Strong plant with pineapple-type fruits (2-3 kg). Excellent aroma and taste, suitable for greenhouses, tunnels, and open fields.

Ananas F1: Hybrid with high aroma and sugar content. Oval fruits weigh 2-2.5 kg.

Identification of appropriate bacterial isolates

The PGPR isolates [FZB42 (*Bacillus amyloliquefaciens*), CC378/2 (*Pantoea agglomerans*), and CC44 (*Pseudomonas fluorescens*),] used in the experiment were available in the stocks of Van Yuzuncu Yil University, Faculty of Agriculture, Department of Plant Protection and whose efficacy was determined in previous studies were used.

Growing medium characteristics

In the experiment, peat-perlite mixture was used at a ratio of 3:1 and 72-vials were used as seedling growing medium. [Peat content: EC: 35 mS/m, pH: 5.5-6.5, Fertilizer content: 1.0 kg/m^3; Perlite content: SiO_2 (72.0 - 76.0 %), Al_2O_3 (11.0 - 17.0 %), K_2O (4.0 - 5.0 %), Na_2O (2.9 - 4.0 %), CaO (0.5 - 2.0 %), MgO (0. 1 - 0.5 %), Fe_2O_3 (0.5 - 1.5 %), TiO_2 (0.03 - 0.2 %), MnO_2 (0.03 - 0.1 %), SO_3 (0 - 0.2 %), H_2O (2 - 7 %).]

Location of the Research Site

The research was conducted in the experimental field of Van Yuzuncu Yil University Research and Application Farm in 2016. The field trial was conducted between April 14 and August 12, and the greenhouse trial was conducted between October 2 and December 15. Van province is located in a basin surrounded by mountains to the west of Lake Van in the Eastern Anatolia Region, 1720 m above sea level and 38-25' north latitude and 43-21' east longitude. The trial area is located northeast of Lake Van, approximately 2 km from the lakeshore.

Climate Characteristics of the Research Site

Van has a continental climate with cold and snow-covered winters and cool and dry summers. Being located on the shores of Lake Van makes the climate of the province relatively mild. Monthly climate data for the periods of the study are presented in Table 1.

Table 1

Some Climate Data for Van Province and the 2016/2017 Season (Anonymous, 2018)

Month	**Precipitation (mm)**	**Temperature (°C)**	**Relative Humidity (%)**
	2016-2017	Long Term Means	2016-2017
September	26.5	13.6	17.5
October	88.8	46.8	11.7
November	27.3	47.0	4.2
December	77.0	36.0	-1.85
January	18.5	34.6	-3.2
February	15.3	33.6	-3.5
March	34.7	46.7	3.2
April	60.5	55.9	8.5
May	90.6	45.8	13.9
June	-	18.1	19.5
July	3.3	5.4	23.9
August	3.1	3.7	24.3
Total	442.3	387.2	-
Average	-	-	9.9

Soil characteristics of the research site

Some physical and chemical analyses of the soil samples taken from 0-30 cm from the experimental area where the research was conducted were carried out in Van Commodity Exchange Laboratory and the results of the analysis are shown in Table 2.

Table 2

Field and Greenhouse Parcels' Soil Analysis Results

Field soil Analyses	**Results**	**Status**
Potassium (K_2O)	131.7918	High
Phosphorus (P_2O_5)	6.6983	Medium
Lime (%)	7.3429	Moderately calcareous
Organic Matter (%)	0.5039	Very low
Total Salt (%)	0.0060	Salt-free
pH	7.16	Slightly alkaline
Saturation (%)	27	Sandy

METHODS

Plant cultivation

For the field trial, six melon varieties, three different PGPR isolates, and a control were sown in 72-well trays on April 25, 2016. Twelve seedlings per plot were planted at 120 cm by 60 cm spacing, resulting in 24 treatments with three replications on May 28, 2016. As base fertilizer only, 25 kg da^{-1} Diammonium phosphate was applied.

PGPR Applications

Each root bacterial isolate was grown in KB medium for 48 hours at 24°C. Bacterial cultures were suspended with 1.5% CMC. PGPR treatments were performed one week apart, starting at seedling emergence. At seedling emergence, a concentration of 10^9 cfu/ml was applied to the roots by inoculation.

Determination of Plant Growth and Fruit Parameters

Plant growth and fruit parameters were evaluated in mid-August on the middle six plants from each plot. Leaf length was measured on the leaf blade at the 4^{th} node using a ruler. Fresh leaf weight was recorded with a precision balance (±0.1g), while leaf dry weight was obtained after drying the leaves to constant weight and measured using a precision balance (±0.01g). Leaf petiole length was assessed with a ruler, and petiole width was determined with a digital caliper. The average number of branches was determined by counting, and the total branch length was measured with a ruler. Main stem thickness at the 4^{th} node was recorded using a digital caliper. Average fruit weight was measured with a precision balance (±1g), and the average number of fruits was determined by counting. Fruit stalk length, fruit diameter, and length were measured with a digital caliper and a ruler, respectively. Fruit flesh and rind thickness were also measured using a digital caliper. Soluble solid content (°Brix) in the fruit juice were measured using a hand refractometer, and the fruit juice pH was determined with a pH meter.

Statistical analysis

The data obtained from Randomized Blocks experimental design were analyzed using analysis of variance (one-way ANOVA) within the SPSS software package (IBM SPSS Statistics 21.0) according to the randomized block experimental design. The means were separated by "Duncan Multiple Comparison Test".

RESULTS AND DISCUSSION

Effects of PGPR Applications on Leaf Petiole Length of Different Melon Cultivars

Significant differences ($P \leq 0.001$) were found between PGPR applications and melon varieties for average leaf petiole length, as shown in Table 3. All PGPR isolates significantly affected the average leaf petiole length compared to the control. The control group's average leaf petiole length was 78.34 cm, whereas the highest average length (88.47 cm) was observed with the FZB42 PGPR isolate. The FZB42 was followed by CC44 with an average length of 86.57 cm and CC37/2 with 84.75 cm.

Statistical differences ($P \leq 0.001$) were also found among the melon varieties. The lowest average leaf petiole length (82.14 cm) was recorded in the Lokum F1 variety, while the highest (86.83 cm) was observed in the Ananas variety. Other varieties such as BT Akhisar, Kırkağaç 637, Napolyon F1, and Lokma F1 followed in decreasing order.

The interaction between melon varieties and PGPR was also significant ($P \leq 0.01$). The highest values (91.29 cm and 91.11 cm) were observed in the Ananas variety with CC44 and FZB42 applications, respectively. The lowest value (77.22 cm) was found in the Kırkağaç 637 variety under control conditions.

The study demonstrated that PGPR applications increased leaf petiole length between 8.1% to 12.9% compared to control treatments. The studies frequently report the use of PGPR strains as microbial fertilizers and biological control agents. For instance, Ji *et al*. (2006) found that some PGPR strains improved disease resistance in tomato plants against P. s. pv. *tomat*o and X. a. pv. *vesicatoria*, suggesting that combining rhizosphere and leaf applications of PGPR strains yielded better results in field conditions.

Table 3

Effects of PGPR Applications on Leaf Length of Different Melon Cultivars (cm)

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	77.22 k**	78.52 ı-k	79.29 ı-k	78.11 jk	78.49 ı-k	78.45 ı-k	78.34 D***
CC37/2	87.67 a-e	84.85 d-h	83.90 e-h	83.42 f-h	82.23 g-ı	86.49 b-f	84.75 C
CC44	88.85 a-d	89.48 a-c	83.17 f-h	84.85 e-h	81.73 h-j	91.29 a	86.57 B
FZB42	87.75 a-e	89.24 a-c	90.28 ab	86.30 b-f	86.10 c-g	91.11 a	88.47 A
MEAN	85.37AB***	85.52 AB	84.16 BC	83.17 CD	82.14 D	86.83 A	

***: Significant at P≤0.001 level; **: Significant at P≤0.01 level;

Effects of PGPR Applications on Leaf Fresh Weight of Different Melon Cultivars

The effects of PGPR applications on average leaf fresh weight are shown in Table 4. Significant differences (P≤0.001) were observed among the PGPR applications. All PGPR isolates significantly affected the average leaf fresh weight compared to the control. The average leaf fresh weight in the control group was 9.53 g, while the highest average (10.75 g) was observed with the CC44 PGPR isolate. This was followed by FZB42 with 10.72 g and CC37/2 with 10.29 g.

No significant differences were found among melon varieties regarding leaf fresh weight. However, the lowest average leaf fresh weight (9.98 g) was found in the Kırkağaç 637 variety, while the highest (10.61 g) was observed in the Napolyon F1 variety. No significant differences were observed in the interaction between melon varieties and PGPR applications.

Overall, PGPR applications increased leaf fresh weight by 7.9% to 12.8% compared to the control treatments. In line with these findings, the study by Kokalis-Burelle *et al.* (2003) reported that PGPR applications increased leaf fresh weight and improved plant growth and quality in melon and watermelon plants.

Table 4

Effects of PGPR Applications on Leaf Fresh Weight of Different Melon Cultivars (g)

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	9.01 ns	9.42	10.34	9.47	9.38	9.58	9.53 C***
CC37/2	9.60	10.35	10.60	10.63	9.93	10.57	10.29 B
CC44	10.62	10.65	10.60	11.05	10.90	10.70	10.75 A
FZB42	10.68	10.48	10.91	10.42	10.94	10.92	10.72 A
MEAN	9.98 ns	10.22	10.61	10.40	10.29	10.44	

***: Significant at P≤0.001 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Leaf Dry Weight of Different Melon Cultivars

The effects of PGPR applications on average leaf dry weight in different melon cultivars are presented in Table 5. Significant differences (P≤0.05) were found among PGPR applications. All PGPR isolates significantly increased the average leaf dry weight compared to the control, which had an average of 3.08 g. The highest leaf dry weight (3.48 g) was observed with the CC37/2 isolate, followed by CC44 (3.35 g) and FZB42 (3.30 g). No significant differences were found among melon varieties. The lowest average leaf dry weight (3.14 g) was observed in the Napolyon F1 variety, while the highest (3.41 g) was in the Lokum F1 variety. PGPR applications increased leaf dry weight by 7.1% to 12.9%. Previous studies have shown that PGPR applications can enhance plant growth and quality, including increasing fresh and dry weights in melon and watermelon seedlings (Kokalis-Burelle *et al*., 2003).

Table 5

Effects of PGPR Applications on Leaf Dry Weight of Different Melon Cultivars (g)

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	2.89 ns	3.10	2.95	3.31	3.19	3.04	3.08 B*
CC37/2	3.73	3.52	3.08	3.38	3.84	3.37	3.48 A
CC44	3.19	3.64	3.35	3.20	3.29	3.43	3.35 A
FZB42	3.41	3.27	3.19	3.30	3.35	3.32	3.30 AB
MEAN	3.30 ns	3.38	3.14	3.29	3.41	3.29	

*: Significant at P≤0.05 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Leaf Petiole Length of Different Melon Cultivars

The impact of PGPR applications on average leaf petiole length is presented in Table 6. No significant differences were found among the melon cultivars regarding leaf petiole length. The lowest mean petiole length was observed in the Ananas cultivar (102.33 mm), while the highest was found in the Napolyon F1 cultivar (105.25 mm). PGPR applications increased (P≤0.001) petiole lengths by 7.6% to 8.3%, suggesting the benefits of PGPR in enhancing growth parameters in vegetables. Previous studies have reported similar positive effects of PGPR on plant height, stem diameter, root length, and seedling growth in Cucurbits and Solanaceous crops (Kokalis-Burelle *et al*., 2002; Garcia *et al*., 2003).

Table 6

Effects of PGPR Applications on Leaf Petiole Length of Different Melon Cultivars (cm)

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	95.33 ns	98.33	99.00	99.00	97.33	96.33	97.55 B***
CC37/2	105.66	105.00	112.00	102.33	105.33	100.00	105.05 A
CC44	104.33	104.00	106.00	108.00	105.66	106.00	105.66 A
FZB42	105.33	101.33	104.00	109.00	106.00	107.00	105.44 A
MEAN	102.66 ns	102.16	105.25	104.58	103.58	102.33	

***: Significant at P≤0.001 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Leaf Petiole Thickness of Different Melon Cultivars

The effects of PGPR applications on leaf petiole thickness in different melon cultivars as presented in Table 7. No significant differences were found among PGPR treatments; however, all PGPR isolates notably affected petiole thickness compared to the control. The control had an average thickness of 3.33 mm, while the highest thickness was observed with the CC37/2 and FZB42 isolates (3.66 mm), followed by the CC44 isolate (3.44 mm). Among cultivars, no s significant differences were detected. The Kırkağaç 637 cultivar had the lowest average petiole thickness (3.41 mm), while BT Akhisar, Lokma F1, and Ananas had the highest thickness (3.58 mm). The cultivar x PGPR interaction was also insignificant.

Table 7

Effects of PGPR Applications on Leaf Petiole Thickness of Different Melon Cultivars (mm)

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	3.00 ns	3.66	3.33	3.33	3.00	3.66	3.33 ns
CC37/2	3.66	3.66	3.66	3.66	4.00	3.33	3.66
CC44	3.66	3.33	3.33	3.66	3.00	3.66	3.44
FZB42	3.33	3.66	3.66	3.66	4.00	3.66	3.66
MEAN	3.41 ns	3.58	3.50	3.58	3.50	3.58	

ns: not significant, there is no statistical difference between means

Effects of PGPR Applications on Average Number of Branches of Different Melon Cultivars

The effects of PGPR applications on the average branch number of different melon cultivars are shown in Table 8. Significant differences (P≤0.001) were found among PGPR treatments, with all isolates significantly increasing branch numbers compared to the control. The control had an average of 4.17 branches, while the highest number (5.05) was observed with the FZB42 isolate, followed by CC44 (4.83) and CC37/2 (4.17). No significant differences were found among cultivars. The BT Akhisar cultivar had the lowest average branch number (4.50), while Lokum F1 had the highest (4.83). The cultivar x PGPR interaction was not statistically significant.

Table 8

Effects of PGPR Applications on Average Number of Branches of Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	4.00 ns	3.67	5.00	3.67	4.67	4.00	4.17 B***
CC37/2	4.67	4.67	5.33	4.33	4.67	5.00	4.78 A
CC44	5.33	4.67	4.67	5.00	4.67	4.67	4.83 A
FZB42	4.67	5.00	5.33	5.33	5.33	4.67	5.05 A
MEAN	4.67 ns	4.50	4.59	4.59	4.83	4.59	

***: Significant at P≤0.001 level; ns: not significant, there is no statistical difference

PGPR applications increased branch numbers by 14.6% to 21.1%. Similar studies, such as Köse (2003), found that bacterial applications significantly increased runner numbers in Selva strawberry cultivars. Another study by Gholami *et al*. (2012) reported that PGPR such as Azospirillum and Azotobacter significantly enhance plant growth parameters, with observed increases in dry weights of leaf, stem, and grain, as well as total biomass, demonstrating the potential of PGPR in improving plant health and productivity under field conditions. The supportive effects of bacterial inoculation on plant growth and development were notable.

Effects of PGPR Applications on Total Branch Length of Different Melon Cultivars

The effects of PGPR applications on the average total branch length of different melon cultivars are shown in Table 9. Significant differences (P≤0.001) were found among PGPR treatments, with all isolates significantly increasing total branch length compared to the control. The control had an average total branch length of 78.61 cm, while the highest was 89.00 cm with the FZB42 isolate, followed by CC44 at 87.11 cm and CC37/2 at 86.28 cm.

No significant differences were found among cultivars. The BT Akhisar cultivar had the lowest average total branch length (82.08 cm), while Napolyon F1 had the highest (86.67 cm). This was followed by Lokma F1, Lokum F1, Ananas, and Kırkağaç in terms of lower total branch length. The cultivar x PGPR interaction was also insignificant. PGPR applications increased average total branch length by 9.7% to 13.2%. Similar studies have shown that PGPR applications improve various growth parameters and plant development. For instance, Ibiene *et al*. (2012) reported enhanced plant growth in tomato seedlings, while Garcia *et al*. (2003) found increased seedling growth in tomatoes and peppers.

Table 9

Effects of PGPR Applications on Total Branch Length (cm) of Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	74.66 ns	79.00	80.00	77.00	80.00	81.00	78.61B***
CC37/2	84.33	86.67	88.33	86.00	85.67	86.67	86.28A
CC44	83.00	87.67	90.00	90.33	86.00	85.67	87.11A
FZB42	86.33	85.33	88.33	92.00	91.33	90.67	89.00A
MEAN	82.08 ns	84.67	86.67	86.33	85.75	86.00	

***: Significant at P≤0.001 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Main Stem Thickness of Different Melon Cultivars

The effects of PGPR applications on the average main stem thickness of different melon cultivars are presented in Table 10. Statistically significant differences (P≤0.001) were found among PGPR treatments, with all isolates significantly increasing the main stem thickness compared to the control. The control had an average main stem thickness of 10.28 mm, while the highest thickness was 11.23 mm with the FZB42 isolate, followed by CC44 at 11.09 mm and CC37/2 at 11.06 mm.

Among cultivars, no significant differences were observed. However, the Kırkağaç 637 cultivar had the lowest average main stem thickness (10.80 mm), and Napolyon F1 had the highest (11.05 mm).

The cultivar x PGPR interaction was statistically significant (P≤0.001). The highest value was obtained with the Lokma F1 x FZB42 PGPR combination (11.69 mm), while the lowest values were observed with Napolyon F1 and Lokma F1 in the control group (10.14 mm and 10.17 mm, respectively).

PGPR applications increased the average main stem thickness by 7.5% to 9.2%. Similar studies have shown positive effects of PGPR on plant growth. For example, Walia *et al*. (2014) reported that *Bacillus subtilis* improved seed germination, stem length, root length, and dry weights in tomatoes. PGPRs enhance plant development by improving nutrient uptake, hormone content, chlorophyll levels, and organic acids. Literature shows that PGPRs can increase yield, root and stem thickness, delay leaf aging, and improve disease resistance (Çakmakçı *et al*., 2005; 2007).

Table 10

Effects of PGPR Applications on Main Stem Thickness (mm) of Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	10.35e-f***	10.14 f	10.17 f	10.31 e-f	10.38 ef	10.35 ef	10.28 B***
CC37/2	11.00 b-d	10.62 d-f	11.23 a-c	11.47 ab	10.95 b-d	11.10 b-d	11.06 A
CC44	11.01 b-d	11.24 a-c	11.35 a-c	10.61 b	11.31 a-c	11.04 b-d	11.09 A
FZB42	10.83 c-e	11.44 ab	11.49 ab	11.69 a	10.61 d-f	11.34 a-c	11.23 A
MEAN	10.80 ns	10.86	11.05	11.01	10.81	10.96	

***: Significant at P≤0.001 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Average Fruit Weight of Different Melon Cultivars

The effects of PGPR applications on the average fruit weight of different melon cultivars are presented in Table 11. Significant differences (P≤0.001) were observed among PGPR treatments, with all isolates significantly increasing fruit weight compared to the control. The control had an average fruit weight of 1490 g, while the highest average fruit weight was 1817 g with the FZB42 isolate, followed by CC44 at 1794 g and CC37/2 at 1781 g.

Table 11

Effects of PGPR Applications on Average Fruit Weight (g) of Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	1340.3 ns	1547.7	1521	1388	1606	1557	1490 B***
CC37/2	1794.0	1953.3	1788	1690	1759	1703	1781 A
CC44	1717.3	1996.0	1873	1601	1827	1751	1794 A
FZB42	1840.7	2193.7	1744	1679	1667	1780	1817 A
MEAN	1773 B**	1922 A	1731 AB	1589 B	1715 AB	1698 B	

***: Significant at P≤0.001 level; **: Significant at P≤0.01 level; ns: not significant, there is no statistical difference

Significant differences (P≤0.01) were also found among melon cultivars. The lowest average fruit weight was observed in the Lokma F1 cultivar (1589 g), while the highest was in the BT Akhisar cultivar (1922 g). Napolyon F1 and Lokum F1 were intermediate, and Ananas and Lokma F1 had the lowest average fruit weight. The cultivar x PGPR interaction was not statistically significant.

PGPR applications increased average fruit weight by 19.5% to 21.9%.. The positive effects of PGPR on plant growth and yield are well-documented, with studies showing benefits across various crops including wheat (de Freitas, 2000), sugar beet and barley (Şahin *et al*., 2004), wheat and spinach (Çakmakçı *et al*., 2007), broccoli (Aydın *et al*., 2012), radish (Güllüce *et al*., 2012), and lettuce (Gül *et al*., 2008).

Effects of PGPR Applications on Average Number of Fruits per Plant for Different Melon Cultivars

Table 12 presents the impact of PGPR applications on the average number of fruits per plant for various melon cultivars. Significant differences (P≤0.001) were observed among PGPR treatments. All PGPR isolates significantly increased the average number of fruits per plant compared to the control. The control treatment resulted in an average of 2.21 fruits per plant, whereas the highest average (3.32 fruits per plant) was achieved with the CC37/2 PGPR isolate. This was followed by FZB42 with an average of 3.09 fruits per plant and CC44 with 3.08 fruits per plant.

Significant differences (P≤0.01) were also found among melon cultivars. The Kırkağaç 637 cultivar had the lowest average number of fruits per plant (2.70), while the Ananas cultivar had the highest (3.11). The remaining cultivars were ranked as follows: Lokum F1, Lokma F1, Napolyon F1, and BT Akhisar. The cultivar x PGPR interaction did not show significant statistical differences.

PGPR applications increased the average number of fruits per plant by 39.3% to 50.2%. Similar results have been reported for other crops. For instance, bacterial inoculants such as Enterobacter have been shown to enhance plant growth and yield in wheat, rice, and sugarcane (Saikia *et al*., 2012; Tahir *et al*., 2013; Karpagam and Nagalakshmi, 2014). Additionally, PGPR application has been noted to support fruit growth and maturation (Ohwaki and Hirata, 1992; Marschner, 1995). While traditional fertilizers containing hormones, amino acids, and minerals can enhance plant development, they can also lead to high chemical usage and increased costs. In contrast, PGPR applications can achieve similar effects with minimal quantities, offering a more cost-effective alternative.

Table 12

Effects of PGPR Applications on Average Number of Fruits per Plant for Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	2.13 ns	2.29	2.13	2.12	2.30	2.30	2.21 C***
CC37/2	2.96	3.39	3.24	3.30	3.37	3.65	3.32 A
CC44	2.65	2.93	3.06	3.17	3.39	3.32	3.08 B
FZB42	3.08	2.86	3.15	2.99	3.27	3.17	3.09 B
MEAN	2.70 C**	2.87 C	2.88 BC	2.89 BC	3.08 AB	3.11 A	

***: Significant at P≤0.001 level; **: Significant at P≤0.01 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Fruit Stalk Length for Different Melon Cultivars

Table 13 details the impact of PGPR applications on the average fruit stem length across various melon cultivars. Significant differences (P≤0.001) were observed among PGPR treatments. All PGPR isolates significantly increased the average fruit stem length compared to the control. The control treatment resulted in an average fruit stem length of 22.83 mm, whereas the highest average (24.94 mm) was achieved with the CC44 PGPR isolate. This was followed by CC37/2 with an average of 24.77 mm and FZB42 with 24.61 mm.

No significant differences were found among the melon cultivars in terms of fruit stem length. However, the Kırkağaç 637 cultivar had the lowest average fruit stem length (23.91 mm), while the Lokma F1 cultivar had the highest average (24.75 mm). The remaining cultivars were ranked as follows: Napolyon F1, Lokum F1, Ananas, and BT Akhisar. However, the cultivar x PGPR interaction did not show significant statistical differences.

PGPR applications increased the average fruit stem length by 7.7% to 9.2%. These results suggest that PGPR treatments can positively influence fruit stem length, aligning with findings from similar studies where PGPR applications have enhanced various plant growth parameters.

Table 13

Effects of PGPR Applications on Fruit Stalk Length (mm) for Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	21.00 ns	22.67	22.67	24.67	23.33	22.67	22.83 B***
CC37/2	24.67	24.33	25.33	25.00	24.67	24.67	24.77 A
CC44	23.00	24.67	25.00	25.00	25.33	24.67	24.94 A
FZB42	25.00	24.67	24.67	24.33	24.00	25.00	24.61 A
MEAN	23.91 ns	24.08	24.41	24.75	24.33	24.25	

***: Significant at P≤0.001 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Fruit Diameter (cm) for Different Melon Cultivars

Table 14 presents the impact of PGPR applications on the average fruit diameter across various melon cultivars. Statistically significant differences (P≤0.05) were observed among PGPR treatments. All PGPR isolates significantly increased the average fruit diameter compared to the control. The control treatment had an average fruit diameter of 14.28 cm, while the highest average diameter (15.34 cm) was achieved with the CC44 PGPR isolate. This was followed by CC37/2 with an average of 15.20 cm and FZB42 with 15.16 cm.

No significant differences were observed among the melon cultivars regarding fruit diameter. However, the Ananas cultivar had the smallest average fruit diameter (14.65 cm), while the Napolyon F1 cultivar had the largest average (15.27 cm). The remaining cultivars were ranked as follows: Kırkağaç 637, BT Akhisar, Lokma F1, and Lokum F1. The cultivar x PGPR interaction did not show significant statistical differences.

PGPR applications increased the average fruit diameter by 6.1% to 7.4%. These results are consistent with similar studies where PGPR applications have enhanced fruit size and growth parameters in various crops, including tomatoes (Ibiene *et al*., 2012) and peppers (Garcia *et al*., 2003).

Table 14

Effects of PGPR Applications on Fruit Diameter (cm) for Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	13.86 ns	14.28	15.02	14.39	14.28	13.36	14.28 B*
CC37/2	15.97	15.34	15.55	14.92	14.70	14.70	15.20 A
CC44	15.66	15.44	15.66	15.23	14.81	15.23	15.34 A
FZB42	15.55	15.55	15.23	14.70	14.81	15.13	15.16 A
MEAN	15.26 ns	15.15	15.36	14.81	14.65	14.73	

*: Significant at P≤0.05 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Fruit Length for Different Melon Cultivars

Table 15 illustrates the impact of PGPR applications on the average fruit length across various melon cultivars. No significant differences were found among the PGPR treatments in terms of fruit length. The control application resulted in an average fruit length of 15.88 cm. The highest average fruit length was recorded with the CC44 PGPR isolate at 17.11 cm. This was followed by CC37/2 with an average of 16.80 cm and FZB42 with 16.77 cm.

Significant differences (P≤0.05) were observed among the melon cultivars. The Napolyon F1 cultivar had the smallest average fruit length at 14.95 cm, while the Lokma F1 cultivar had the largest average fruit length at 17.50 cm. The remaining cultivars were ranked as follows: Lokum F1, BT Akhisar, Ananas, and Kırkağaç 637. No significant differences were detected in the cultivar x PGPR interaction.

In the present study, PGPR applications did not show significant differences in their effects on fruit length. This result is consistent with another study by Naidu *et al*. (2013), where these researchers demonstrated that the foliar application of microbial-enriched compost tea significantly enhances fruit quality traits in muskmelon, including increased fruit size and mesocarp thickness.

Table 15

Effects of PGPR Applications on Fruit Length (cm) for Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	14.33 ns	16.00	15.00	17.00	16.00	17.00	15.88 ns
CC37/2	15.66	17.66	14.83	18.33	17.33	17.00	16.80
CC44	17.66	18.00	14.33	18.00	18.00	16.66	17.11
FZB42	18.00	17.00	15.66	16.66	17.66	15.66	16.77
MEAN	16.41 A*	17.16 A	14.95 B	17.50 A	17.25 A	16.58 A	

*: Significant at P≤0.05 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Fruit Flesh Thickness for Different Melon Cultivars

Table 16 shows the impact of PGPR applications on the average fruit flesh thickness across various melon cultivars. Significant differences (P≤0.01) were found among the PGPR treatments. All PGPR isolates significantly increased the average fruit flesh thickness compared to the control. The control application had an average fruit flesh thickness of 3.11 cm. The highest average fruit flesh thickness was recorded with the CC44 PGPR isolate at 3.66 cm. This was followed by FZB42 with 3.55 cm and CC37/2 with 3.50 cm.

Among the melon cultivars, no significant differences were observed in fruit flesh thickness. However, the lowest average fruit flesh thickness was found in the Kırkağaç 637 cultivar (3.41 cm), while the highest was in the Lokma F1 cultivar (3.58 cm). The remaining cultivars followed in the order: Napolyon F1, BT Akhisar, Lokum F1, and Ananas. No significant statistical differences were detected in the cultivar x PGPR interaction.

In this study, PGPR applications increased the average fruit flesh thickness by 12.5% to 17.6%. This result is consistent with another study by Naidu et al. (2013), which reported an 8.81% increase in fruit firmness and a 7.50% increment in mesocarp size, highlighting the positive impact of microbial treatments on fruit flesh characteristics.

Table 16

Effects of PGPR Applications on Fruit Length (cm) for Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	3.00 ns	3.00	3.00	3.00	3.33	3.33	3.11 B**
CC37/2	3.66	3.33	3.66	3.66	3.33	3.33	3.50 A
CC44	3.33	3.66	4.00	4.00	3.66	3.33	3.66 A
FZB42	3.66	4.00	3.33	3.66	3.00	3.66	3.55 A
MEAN	3.41 ns	3.50	3.50	3.58	3.33	3.41	

**: Significant at P≤0.01 level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Fruit Rind Thickness for Different Melon Cultivars

Table 17 presents the effects of PGPR applications on average fruit rind thickness across various melon cultivars. No significant differences were found among the PGPR treatments. The control application resulted in an average rind thickness of 4.24 mm. The highest average rind thickness was observed with the CC44 PGPR isolate at 4.30 mm, followed by CC37/2 with 4.28 mm and FZB42 with 4.25 mm.

Among the melon cultivars, no significant differences were found in rind thickness. However, the lowest average rind thickness was in the Kırkağaç 637 cultivar (4.21 mm), while the highest was in the Lokum F1 cultivar (4.30 mm). The other cultivars followed in order: Lokma F1, BT Akhisar, Napolyon F1, and Ananas. No significant differences were detected in the cultivar x PGPR interaction.

Table 17

Effects of PGPR Applications on Fruit rind width (mm) for Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	4.16 ns	4.19	4.19	4.22	4.42	4.24	4.24 ns
CC37/2	4.20	4.33	4.37	4.28	4.23	4.26	4.28
CC44	4.26	4.48	4.24	4.29	4.25	4.28	4.30
FZB42	4.24	4.15	4.22	4.36	4.33	4.20	4.25
MEAN	4.21 ns	4.29	4.25	4.29	4.30	4.24	

ns: not significant, there is no statistical difference

Effects of PGPR Applications on Soluble Solid Content for Different Melon Cultivars

Table 18 presents the effects of PGPR applications on the soluble solid content (SSC) of various melon cultivars. No significant differences were found among the PGPR treatments. The control application resulted in an average SSC of 10.06 Brix. The highest average SSC was observed with the CC37/2 PGPR isolate at 10.22 Brix, followed by CC44 with 10.18 Brix and FZB42 with 10.00 Brix.

Statistical analysis revealed significant differences among melon cultivars ($P \leq 0.01$). The lowest average SSC was found in the Lokma F1 cultivar (9.78 Brix), while the highest was in the BT Akhisar cultivar (10.33 Brix). The other cultivars followed in order: Napolyon F1, Lokum F1, Ananas, and Kırkağaç 637. However, no significant statistical differences were detected in the cultivar x PGPR interaction.

In this study, PGPR applications did not result in significant differences in SSC compared to the control. However, Naidu *et al*. (2013) demonstrated that the application of microbial-enriched compost tea resulted in a 16.21% increase in total soluble solids concentration (SSC), indicating improved fruit sweetness and quality.

Table 18

Effects of PGPR Applications on Soluble Solid Content (Brix) of Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	10.43 ns	10.16	10.17	9.50	10.14	9.94	10.06 ns
CC37/2	10.40	10.50	10.20	9.90	10.27	10.05	10.22
CC44	9.83	10.50	10.13	10.13	10.23	10.22	10.18
FZB42	9.76	10.17	10.32	9.60	9.90	10.24	10.00
MEAN	10.10 A**	10.33 A	10.20 A	9.78 B	10.13 A	10.11 A	

**: Significant at $P \leq 0.01$ level; ns: not significant, there is no statistical difference

Effects of PGPR Applications on Fruit pH of Different Melon Cultivars

Table 19 presents the effects of PGPR applications on the pH levels of various melon cultivars. No significant differences were observed among the PGPR treatments. The control group had an average pH of 6.70. The highest average pH was found with the control group (PGPR0) at 6.70, followed by

CC37/2 with 6.59 and CC44 with 6.58.

Table 19

Effects of PGPR Applications on Fruit pH of Different Melon Cultivars

PGPR	CULTIVARS						MEAN
	Kırkağaç 637	BT-Akhisar Topan	Napolyon F1	Lokma F1	Lokum F1	Ananas	
CONTROL	6.76 ns	6.46	6.66	6.91	6.67	6.74	6.70 ns
CC37/2	6.45	6.62	6.48	6.69	6.55	6.76	6.59
CC44	6.50	6.56	6.52	6.64	6.60	6.66	6.58
FZB42	6.35	6.35	6.58	6.53	6.78	6.74	6.55
MEAN	6.52 ns	6.50	6.56	6.69	6.65	6.73	

ns: not significant, there is no statistical difference

Statistical analysis showed significant differences among melon cultivars. The lowest average pH was recorded for the BT Akhisar cultivar at 6.50, while the highest was found in the Ananas cultivar at 6.73. The other cultivars followed in the order: Lokma F1, Lokum F1, Napolyon F1, and Kırkağaç 637. No significant statistical differences were detected in the cultivar x PGPR interaction.

In the study by Murgese *et al*. (2020), the application of a consortium of plant growth-promoting bacteria (PGPB) to Barattiere (*Cucumis melo* L.) plants showed significant improvements in fruit yield, early maturity, and physiological parameters, even when used with reduced doses of mineral fertilizers. This study highlights the potential of PGPR to enhance plant growth and nutrient uptake by upregulating genes involved in nitrogen, iron, and phosphorus transport. Similar to these findings, our study demonstrates that PGPR applications can positively impact yield and quality traits in melons, suggesting that integrating PGPR could reduce the need for full fertilizer doses while maintaining or improving crop performance. This approach aligns with sustainable agricultural practices by minimizing chemical inputs and supporting environmental and economic benefits.

In the study by Altuntaş and Kutsal (2022), the impact of various plant growth-promoting rhizobacteria (PGPR) on melon development and fruit quality was evaluated under both irrigated and non-irrigated conditions. Their findings demonstrated that while PGPR treatments did not significantly affect most fruit quality parameters, *Bacillus subtilis* notably improved total soluble solids (TSS) in Kırkağaç 637 melons grown under non-irrigated conditions. This highlights the potential of specific PGPR strains to enhance fruit quality, particularly in challenging growing environments. In our study, similar PGPR applications showed varying effects on melon yield and quality traits, suggesting that the choice of PGPR strain and application conditions can significantly influence crop performance. Integrating PGPR into melon cultivation could be a valuable strategy for optimizing fruit quality and yield, especially in regions with limited irrigation resources.

CONCLUSION

The present study evaluated the effects of different plant activators on various melon cultivars (Kırkağaç 637, BT Akhisar Topan, Napolyon F1, Lokma F1, Lokum F1, and Ananas) in Van conditions, focusing on plant growth, yield, and quality. The results revealed significant improvements in parameters such as stem diameter, stem length, leaf fresh and dry weight, average fruit weight, fruit width, fruit length, petiole length, average number of branches, average branch length, and average number of fruits per plant due to the application of CC37/2, CC44, and FZB42. However, no statistically significant effects were observed for soluble solid content (SCC), pH, rind thickness, and petiole thickness in relation to PGPR applications.

The application of CC37/2, CC44, and FZB42 bacteria improved plant growth and fruit quality by affecting the mineral content, amino acids, organic acids, and hormone levels in the melons. This indicates that PGPRs may enhance these compounds, which in turn boosts plant development and fruit quality. These findings suggest that PGPRs could offer significant economic and environmental benefits in vegetable cultivation, with minimal impact on human health. Further research should explore the use of these bacteria as biofertilizers in commercial vegetable production and their effects on other vegetable species.

Additionally, the highest average branch number was observed in the Ananas cultivar, while BT Akhisar had the highest average fruit weight, and Ananas also showed the highest average fruit yield. It is recommended that specialized sales outlets or distributors be established to make these plant activators accessible to producers and provide agricultural extension services on their use. Successful isolates such as CC44/2, CC44, and FZB42 should be formulated and tested for practical application. Furthermore, producers should be encouraged to adopt integrated pest management practices and monitor soil health regularly to complement the benefits of PGPR applications. Developing and implementing educational programs on the benefits and techniques of using PGPRs can enhance their adoption and effectiveness. Collaborative efforts with agricultural extension services and research institutions can also facilitate the dissemination of best practices and innovations in PGPR use.

Ethical Statement

The article is adapted from the first author's M.Sc. thesis. The authors would like to thank Dr. A. Akkopru, Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Plant Protection, for the PGPR supply.

Author Contributions

Research Design (CRediT 1) Author 1 (50%) - Author 2 (50%)

Data Collection (CRediT 2) Author 1 (70%) - Author 2 (30%)

Research - Data Analysis - Validation (CRediT 3-4-6-11) Author 1 (50%) - Author 2 (50%)

Writing the Article (CRediT 12-13) Author 1 (60%) - Author 2 (40%)

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Sustainable Development Goals (SDG)

12 Responsible Production and Consumption

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Alfalfa Silage Utilisation and Alfalfa Fermentation in Cattle: A Bibliometric Analysis

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ABSTRACT

Alfalfa silage is widely used in the ration of animals. The aim of this study was to determine the publication trends and trending topics of studies on alfalfa silage utilisation and alfalfa fermentation (CAU) in cattle based on the data obtained from the Web of Science (WoS) Core Collection database. The Biblioshiny package, VOSviewer viewing programme and Excel 2022 were used for analysis. A total of 399 documents related to the researched topic were retrieved from WoS from 1980 to 2024. 1203 authors were included in the study. The average citation rate per article is 38.36. The number of articles with a single author is 16. Most of the publications on CAU were found in English language. It was found that the journal that published the most articles was Journal of Dairy Science (198), the most prolific author was GA. Broderick (27), the most cited author was GA. Broderick (2254), the most cited document author was MS. Allen (1073), the most frequently used keyword was “dairy cow”, and the most cited country was USA (459). When the results of the trend topic analysis are analysed, it can be said that the topics on increasing silage quality and nutrient content have come to the forefront in recent researches. This study can provide detailed information about the situation in the current literature in the field of CAU.

Sığırlarda Yonca Silajı Kullanımı ve Yonca Fermantasyonu: Bir Bibliyometrik Analiz

Makale Bilgisi

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Anahtar Kelimeler:
Alfalfa silage,
Alfalfa fermentation,
Cow,
Cattle,
Bibliometric analysis.

ÖZET

Yonca silajı hayvanların rasyonunda yaygın olarak kullanılmaktadır. Bu çalışmada, Web of Science (WoS) Çekirdek Koleksiyonu veri tabanından elde edilen verilere dayanarak sığırlarda yonca silajı kullanımı ve yonca fermantasyonu (CAU) ile ilgili çalışmaların yayın eğilimlerini ve trend konularını belirlemek amaçlanmıştır. Analiz için Biblioshiny paketi, VOSviewer görüntüleme programı ve Excel 2022’den yararlanılmıştır. WoS’dan 1980'den 2024'e kadar araştırılan konu ile ilgili toplam 399 doküman alınmıştır. Çalışmaya 1203 yazar dahil edilmiştir. Makale başına düşen ortalama atıf oranı 38,36’dır. Tek yazarlı makale sayısı 16’dır. CAU konusunda en fazla İngilizce dilinde yayına ulaşılmıştır. En fazla makale yayınlayan derginin Journal of Dairy Science (198), en üretken yazarın GA. Broderick (27), en çok atıf alan yazarın GA. Broderick (2254), en çok atıf alan belge yazarının MS. Allen (1073), en sık kullanılan anahtar kelimenin “dairy cow” ve en fazla atıf alan ülkenin ABD (459) olduğu sonuçlarına ulaşılmıştır. Trend konu analizi sonuçları incelendiğinde ise son dönemdeki araştırmalarda silaj kalitesinin ve besin içeriğinin artırılmasına yönelik konuların ön plana çıktığı söylenebilir. Bu çalışma CAU alanında mevcut literatürdeki durum hakkında ayrıntılı bilgi sağlayabilir.

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INTRODUCTION

Forages represent the main source of components in dairy cow diets (Hassanat *et al.*, 2013). In animal nutrition, especially in ruminant nutrition, the use of quality roughage is very important to reduce feeding costs (Harmanşah, 2018). Alfalfa has become a popular forage for silage due to its high crude protein (CP) content and dry matter (DM) yield (Beauchemin *et al.*, 2003). In recent years, scientific studies have been carried out on plants with high tolerance to drought and desertification, which can be potential animal feed and can be used in the improvement of pastures (Armağan and Işık, 2022). Alfalfa is highly important for livestock, given its rapid canopy recovery after each harvest, its capacity to withstand extreme temperatures (e.g. cold nights and hot days), its relative tolerance to salinity, its palatability for livestock and its nutritional value (Ferreira *et al.*, 2015). Alfalfa is frequently used in animal husbandry as roughage due to its high nutrient content (Grev *et al.*, 2017). Alfalfa adapts well to different environmental conditions due to its rich and variable genetic basis and has a wide cultivation area (Radović *et al.*, 2009). Physical changes of feeds (such as particle size) can affect rumen digestion, transit rate and microbial protein synthesis and therefore postruminal or total digestion (Yang *et al.*, 2002). Most alfalfa hay fed to dairy cattle has a CP content of 20% or more (on a DM basis) and about 80% is ruminally degradable (Kleinschmit *et al.*, 2007). Silage is the product obtained as a result of anaerobic fermentation of water-soluble carbohydrates to organic acids in forage plants (Zhang *et al.*, 2018). The purpose of ensiling feeds is to preserve the nutritional composition of the starting material (Garcia *et al.*, 1989). Compared to cereal silages, alfalfa silage contains less neutral detergent fibre (NDF) and more CP (Mustafa *et al.*, 2000). Alfalfa silage is widely used in the ration of animals (Bai *et al.*, 2020). Silaging of alfalfa is difficult due to its relatively low content of epiphytic lactic bacteria (LAB) and water-soluble carbohydrate (WSC) (Li *et al.*, 2024). The use of traditional homolactic bacterial inoculants as starters for alfalfa silage is a recommended practice to minimise the loss of nutrients and DM in the early stages of the ensiling process and to ensure rapid fermentation (Schmidt *et al.*, 2009). LAB are considered the most important microorganisms in determining the final performance of silage (Li *et al.*, 2020).

Bibliometric methods are used to provide quantitative analysis of written publications (Ellegaard and Wallin, 2015). Bibliometrics is simply the study and measurement of all forms of written communication and the publication patterns of its authors (Potter, 1981). The obvious advantages of bibliometrics are that it allows scientists to study a specific area of research and draw very useful conclusions by analysing citations, co-citations, word frequency and geographical distribution (Liao *et al.*, 2018). In this study, it is aimed to determine the publication trends and trending topics of CAU-related studies based on the data obtained from WoS.

MATERIALS AND METHODS

WoS (Science Citation Index, Social Sciences Citation Index and Arts and Humanities Citation Index), which encompasses three ISI (Institute for Scientific Information) citation databases, is the standard tool for a significant portion of citation studies worldwide (Meho and Yang, 2007). In this study, WoS was used as the data source.

The documents constituting the dataset of the study were retrieved from the WoS Core Collection database on 14 September 2024. Search strategy: Articles were searched using the keywords TS=(("alfalfa silage" OR "alfalfa fermentation") AND ("cow" OR "cattle")). No restriction was applied in the data category. As a result of the searches, 399 documents were found and these documents were downloaded from the WoS database in BibTeX format. Bibliometric analysis of 399 documents was performed with R programming language.

VOSviewer is a software tool for creating and visualising bibliometric networks (Van Eck and Waltman, 2017). VOSviewer can be used to analyse all kinds of bibliometric network data, such as co-occurrence relationships between scientific terms, citation relationships between publications or journals, and collaboration relationships between researchers (Van Eck and Waltman, 2011). Bibliometrix and Biblioshiny were developed by Italian academic Massimo Aria in R language environment (Xie *et al.*, 2020).

Biblioshiny is an in-package tool designed for non-coders to provide tools for a complete scientometric and bibliometric analysis, offering numerous options categorised by source, author, document, social structure, conceptual structure and intellectual structure (Nasir *et al.*, 2020). In this study, Biblioshiny package, VOSviewer viewing programme and Excel 2022 were used for analysis.

FINDINGS

General information and annual publication output

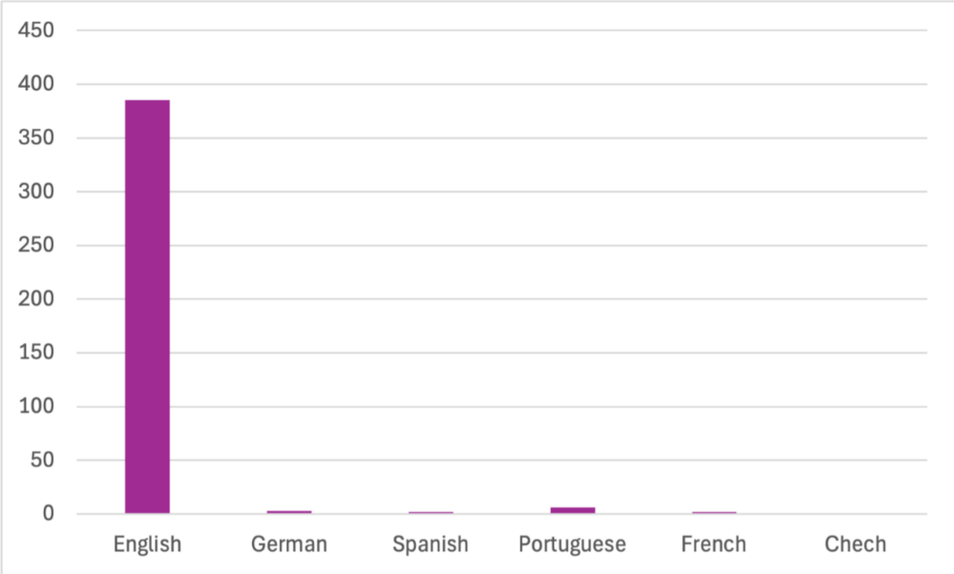
Table 1 shows general information about the data and document types obtained from WoS. A total of 399 documents related to the researched topic were retrieved from WoS from 1980 to 2024. Of these, 367 are articles, 7 are article: proceedings paper, 1 is a correction, 3 are meeting abstracts, 11 are proceedings paper, and 10 are reviews. These documents were taken from 78 different sources. 873 authors included keywords. 1203 authors were included in the study. The average citation rate per article is 38.36. The number of articles with a single author is 16.

Table 1
Main data information

Description	Results
MAIN INFORMATION ABOUT DATA	
Timespan	1980:2024
Sources (Journals, Books, etc)	78
Documents	399
Annual Growth Rate %	5,12
Document Average Age	16,4
Average citations per doc	38,36
References	9527
DOCUMENT CONTENTS	
Keywords Plus (ID)	994
Author's Keywords (DE)	873
AUTHORS	
Authors	1203
Authors of single-authored docs	14
AUTHORS COLLABORATION	
Single-authored docs	16
Co-Authors per Doc	4,4
International co-authorships %	21,3
DOCUMENT TYPES	
article	367
article; proceedings paper	7
correction	1
meeting abstract	3
proceedings paper	11
review	10

Figure 1

Language of publication of documents

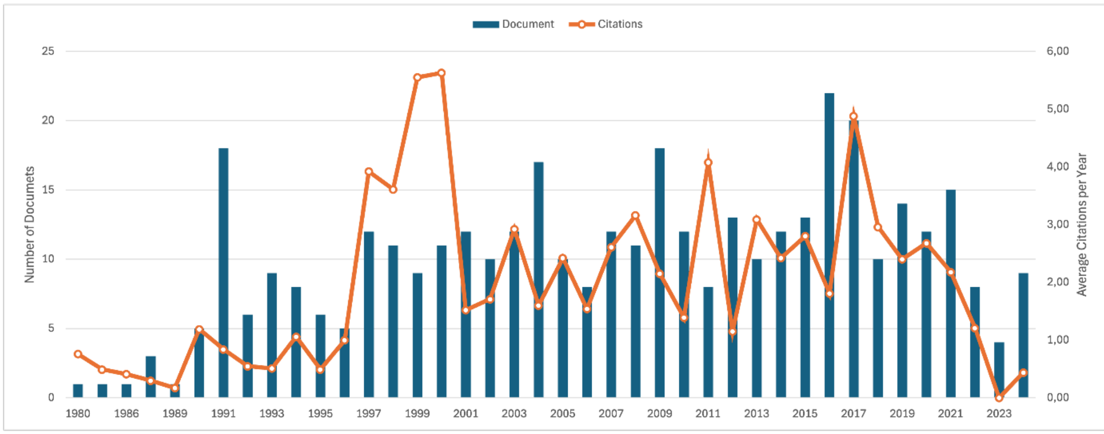


Various languages were used in the results of the outputs. It is noteworthy that most of the results are in English (385). Six are in Portuguese, three in German, two in French, two in Spanish and one in Chech. These results are given in Figure 1.

Figure 2 shows the distribution of citations and articles by year. The general trend increased from 1 article in 1980 to 9 articles in 2024. With 22 articles, the highest number of articles was reached in 2016. Fluctuations in the number of articles and citations are noteworthy. However, the annual growth rate reached 5.12%.

Figure 2

Distribution of annual CAU-related articles and citations from 1980 to 2024



Active Journals

A total of 399 articles were published in 78 journals, and four journals published more than twenty articles on CAU research.

Table 2

Number of publications in journals and some indexes

Source	H-index	G-index	M-index	TC	NP	PYS
JOURNAL OF DAIRY SCIENCE	56	101	1,436	11723	198	1986
CANADIAN JOURNAL OF ANIMAL SCIENCE	16	22	0,356	564	35	1980
ANIMAL FEED SCIENCE AND TECHNOLOGY	15	25	0,441	653	26	1991
JOURNAL OF ANIMAL SCIENCE	15	21	0,429	933	21	1990
ASIAN-AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES	5	8	0,2	77	8	2000
ANIMAL	3	4	0,2	117	4	2010
JOURNAL OF CLEANER PRODUCTION	3	3	0,5	64	3	2019
JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE	3	3	0,15	63	3	2005
LIVESTOCK SCIENCE	3	4	0,176	78	4	2008
AGRICULTURE ECOSYSTEMS \& ENVIRONMENT	2	2	0,222	36	2	2016

TC: Total Citation, NP: Number of Paper, PYS: Publication Year Start

When Table 2 is analysed, Journal of Dairy Science is the journal that publishes the most articles. The number of articles is 198. This journal was followed by "Canadian Journal of Animal Science" (35) and "Animal Feed Science and Technology" (26).

Journal of Dairy Science (JDS), the world's leading general dairy research journal, is the official journal of the American Dairy Science Association. JDS readers represent government, educational and industry organisations in more than 70 countries with interests in biochemistry, genetics, reproduction, food science, microbiology, economics, environment, engineering and nutrition, food safety, processing, physiology, pathology, public health, sanitation and quality assurance.

The Canadian Journal of Animal Science is a quarterly journal that publishes new work in all areas of animal products and animal agriculture, including reproduction and genetics; growth and development; cellular and molecular biology; animal systems modeling; meat science; physiology and endocrinology; non-ruminant nutrition; ruminant nutrition; ruminant behavior, management, and welfare.

Animal Feed Science and Technology is a unique journal of international interest, publishing scientific papers focussing on animal nutrition and feeds.

According to the source local impact total citation index, Journal of Dairy Science was the journal with the highest number of citations in the CAU survey with 11723 total citations. This journal was followed by Journal of Animal Science and Animal Feed Science and Technology with 933 and 653 citations respectively.

Analysis of highly cited documents

Table 3 shows the top ten citation analysis of documents related to CAU research from 1980 to 2024. MS. Allen's paper "Effect of diet on short-term regulation of feed intake by lactating dairy cattle" is the most cited paper with 1073 citations. MS. Allen's article titled "Relationship between fermentation acid production in the rumen and the requirement for physically effective fibre" ranks second with 611 citations. RFD. Valadares's "Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives" is in the third place with 515 citations.

Table 3
Top ten citation analysis of documents related to CAU research.

Paper	DOI	TC	TC per Year
ALLEN MS, 2000, J DAIRY SCI	10.3168/jds.S0022-0302(00)75030-2	1073	42,92
ALLEN MS, 1997, J DAIRY SCI	10.3168/jds.S0022-0302(97)76074-0	611	21,82
VALADARES RFD, 1999, J DAIRY SCI	10.3168/jds.S0022-0302(99)75525-6	515	19,81
BRODERICK GA, 1997, J DAIRY SCI	10.3168/jds.S0022-0302(97)76262-3	416	14,86
DHIMAN TR, 1999, J DAIRY SCI	10.3168/jds.S0022-0302(99)75458-5	334	12,85
JONKER JS, 1998, J DAIRY SCI	10.3168/jds.S0022-0302(98)75825-4	331	12,26
LEONARDI C, 2003, J DAIRY SCI	10.3168/jds.S0022-0302(03)73634-0	293	13,32
CASTILLO AR, 2000, J ANIM FEED SCI	NA	252	10,08
OLIVEIRA AS, 2017, J DAIRY SCI	10.3168/jds.2016-11815	243	30,38
WHITE SL, 2001, J DAIRY SCI	10.3168/jds.S0022-0302(01)74676-0	236	9,83

TC: Total Citation

Analysis of Keywords and Co-occurrence Network

Figure 3 shows the keyword network analysis in the CAU research area. In the analysis of keywords used in articles, a keyword should be used at least five times (Şahiner-Tufan and Midilli-Sarı, 2024a). Out of a total of 837 keywords, 39 keywords with at least 5 occurrences were analysed. According to the criteria determined in the analysis, 7 separate clusters were formed with 39 keywords, 167 links and a total of 275 link strength.

The size of the circles indicates the most frequently used keywords in the keyword network analysis. Words clustered under the same colour indicate the strength of the relationship (Şahiner-Tufan and Midilli-Sarı, 2024a; Ye *et al.*, 2024). The most frequently used keyword was "dairy cow". This was followed by "alfalfa silage" and "silage".

Words in clusters;

Cluster 1: cellulase, digestibility, essential oil, intake, lactating cow, protein, silage and starch

Cluster 2: dairy cow, feed efficiency, manure, milk yield, nitrogen balance, nitrogen efficiency, nitrogen utilisation and rumen fermentation

Cluster 3: alfalfa silage, corn silage, digestion kinetics, enteric methane, fibre and particle size

Cluster 4: cow, forage, greenhouse gas, methane, milk and rumen

Cluster 5: alfalfa, beef cattle, fatty acids and ruminal fermentation

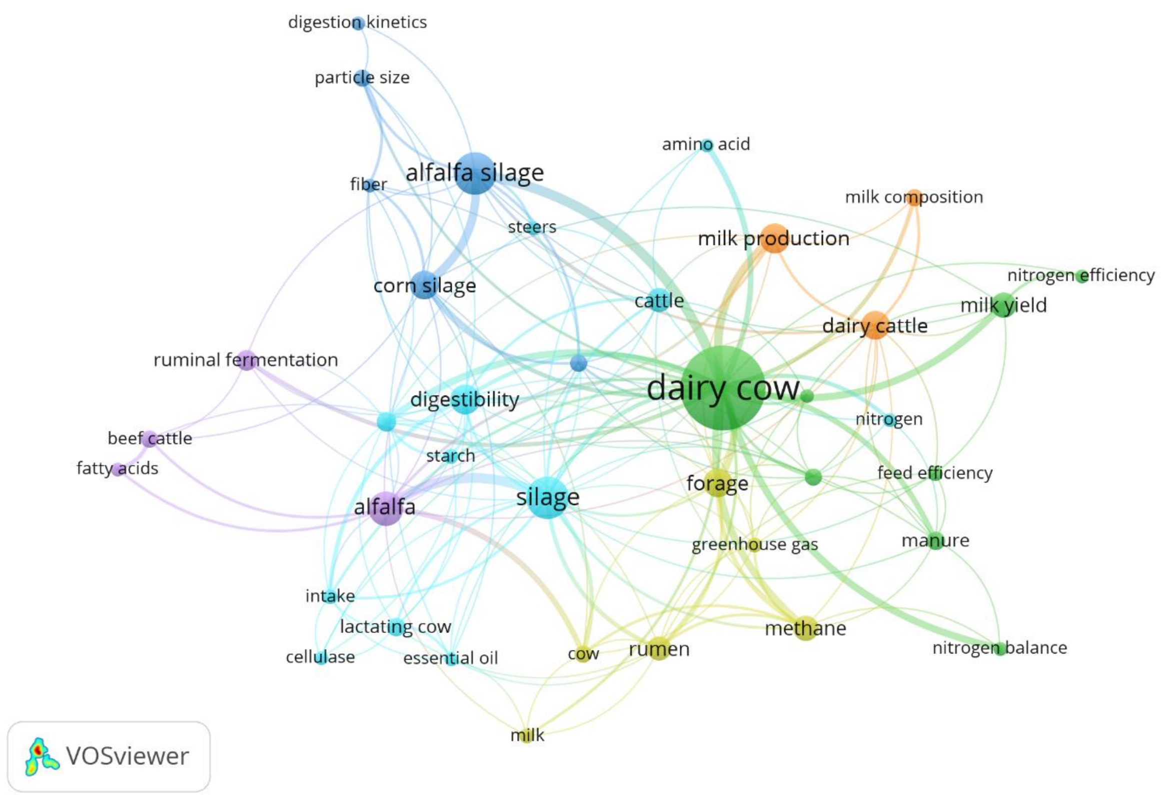
Cluster 6: amino acid, cattle, nitrogen and steers

Cluster 7: dairy cattle, milk composition and milk production keywords.

The keyword "dairy cow" in Cluster 2 is the most frequently used word with 83 total link strength and 26 links. The keyword "silage" in Cluster 1 has 42 total link strength and 24 links. The keyword "alfalfa" in Cluster 5 has 27 total link strength and 15 links.

Figure 3

Network map of co-occurrence between keywords



Analysis Authors

A total of 1203 authors contributed to 399 articles related to CAU research. The number of articles by the most active authors and some indexes are shown in Table 4. GA. Broderick was the most prolific author with 27 articles, followed by KA. Beauchemin (19), MA. Wattiaux (18) and MS. Allen (14). GA. Broderick has the highest H-index (18) and the highest number of citations (2254).

Table 4

Number of publications of authors and some indexes

Author	H-index	G-index	M-index	TC	NP	PYS
BRODERICK GA	18	27	0,643	2254	27	1997
BEAUCHEMIN KA	16	19	0,457	976	19	1990
WATTIAUX MA	13	18	0,382	646	18	1991
ALLEN MS	11	14	0,379	2027	14	1996
BENCHAAR C	10	12	0,556	649	12	2007
KENNELLY JJ	10	13	0,294	500	13	1991
KHORASANI GR	10	10	0,294	458	10	1991
ARMENTANO LE	9	10	0,243	653	10	1988
HASSANAT F	9	10	0,75	424	10	2013
MCALLISTER TA	9	9	0,333	523	9	1998

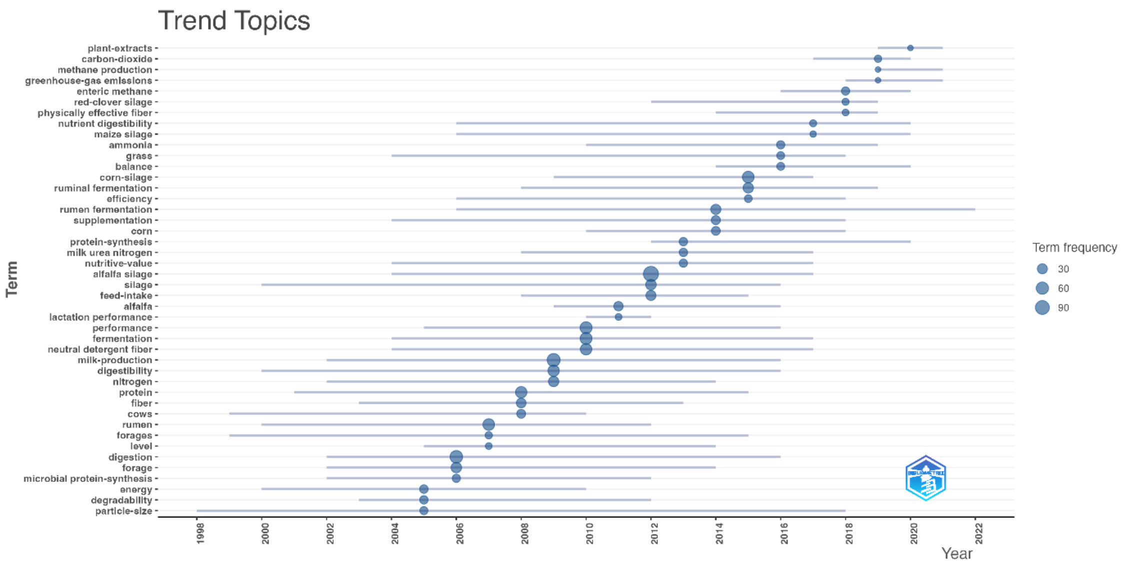
TC: Total Citation, NP: Number of Paper, PYS: Publication Year Start

Trending Topics

Figure 4 shows 45 keywords plus, which are trend topics in 2005-2024. While creating the trend topic graphs, the "word minimum frequency" parameter was taken as five and the "number of words per year" parameter was taken as three. It was determined that the first five keywords that are trend topics in the CAU topic are plant-extracts, carbon-dioxide, methane production, greenhouse-gas emissions and enteric methane. The most frequently used keywords were alfalfa silage, milk production, fermentation, performance and neutral detergent fibre.

Figure 4

Trending topics between 2005-2024

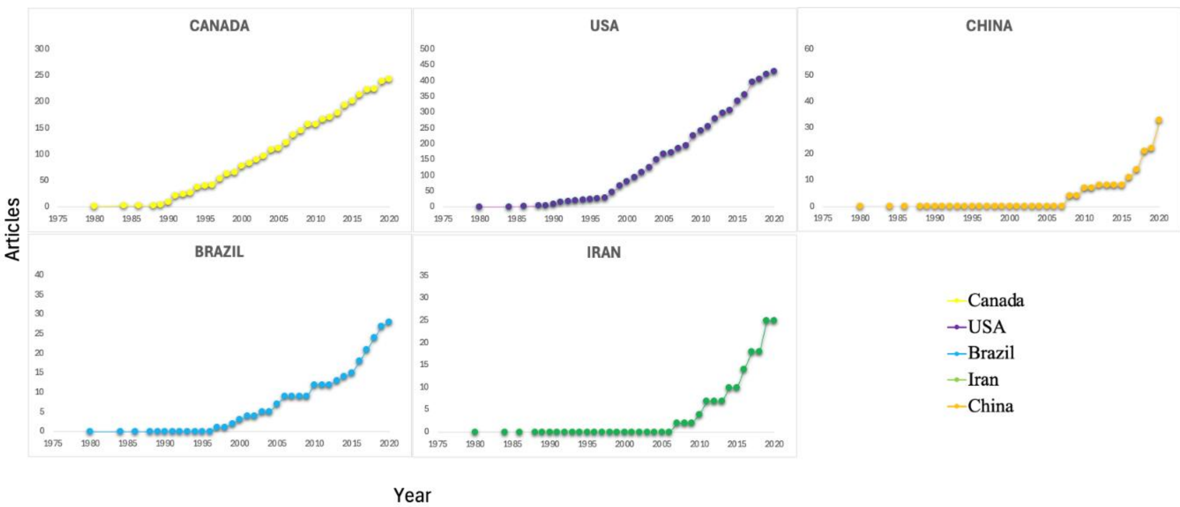


Distribution of Countries

The production of countries in CAU research over time is shown in Figure 5. The USA produced the most articles (459), followed by Canada (255), China (56), Brazil (35) and Iran (32).

Figure 5

Production of countries over time

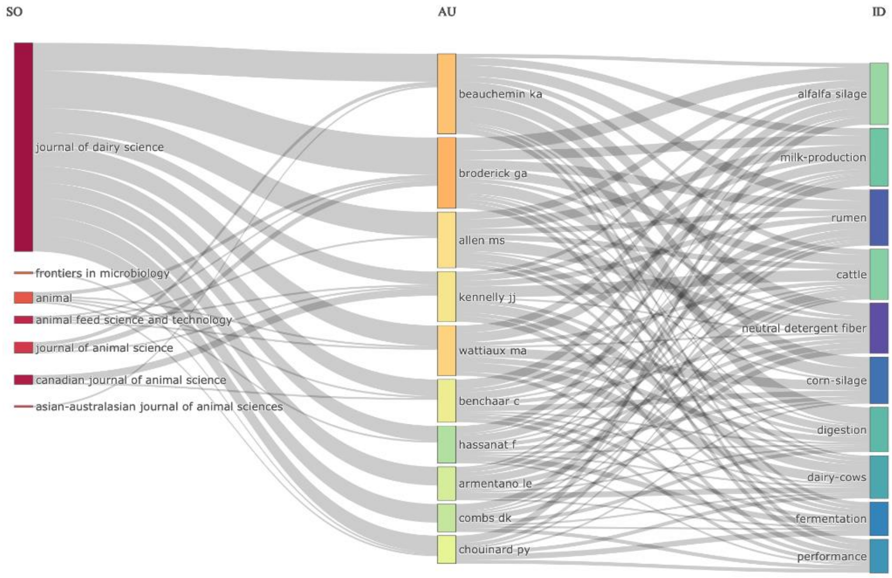


Three Field Graphics

In the three area graph in Figure 6, 10 frequently used keywords plus are given in the right part of the graph. The most frequently used keywords are alfalfa silage, milk production, rumen, cattle, neutral detergent fibre, corn-silage, digestion, dairy cows, fermentation and performance. The word "alfalfa silage" was preferred by most authors. It was mostly used by GA. Broderick. Journal of Dairy Science was the most preferred journal. GA. Broderick and KA. Beauchemin were the authors who preferred "Journal of Dairy Science" most frequently.

Figure 6

Illustration of the relationship between authors, references and keywords with three area graphs



DISCUSSION

Test methods used in veterinary science are not limited and are supported by new methods every day (Selvi, 2024). In this study, the progress of research on alfalfa silage utilisation and alfalfa fermentation in cattle was analysed for more than 40 years from 1980 to 2024. When the distribution of the number of articles according to years and the results of the annual growth rate are analysed, it is thought that the number of studies in this field will increase in a fluctuating manner in the coming years. Out of 399 articles, 16 were single-authored and 383 were multi-authored. According to this result, it can be said that there are many collaborative studies in the field of CAU. When the results of the analysis of journals and countries are analysed, it is seen that the number of citations to Journal of Dairy Science, an American journal, is clearly ahead of other journals. It is also noteworthy that the total number of publications in the USA (459) is higher than other countries. It is thought that this situation is due to the fact that the state is very large, provides serious support to academic studies, hosts many universities and supports the work of talented scientists trained in the field of science and technology in US universities (Şahiner-Tufan and Midilli-Sarı, 2024b). Although MS. Allen and GA. Broderick started their publications on this subject in similar years, the most prolific author is GA. Broderick with 27 papers. However, MS. Allen's article "Effects of diet on short-term regulation of feed intake by lactating dairy cattle" is the leader in this field with 1073 citations.

This article discusses some of the characteristics of dietary components that should be considered when formulating diets for lactating dairy cows and when allocating feed to different animal groups on the farm (Allen, 2000). It serves as a good reference for researchers working in this field. Keywords play a very important role in scientific research as they reflect the main content of the related articles.

Analysing the co-occurrence of keywords allows to determine the closeness and universality of research topics in a scientific field (Ye *et al.*, 2024).

According to the keyword analysis, it is seen that digestibility and milk production are important areas of research on this subject, which include comparative studies with corn silage, which is an important roughage used in animal nutrition as green grass and silage (Erdurmuş, 2023). When the results of the trend topic analysis are analysed, it can be said that the topics on increasing silage quality and nutrient content have come to the forefront in recent researches. There are some limitations in this study. WoS database was used for the research. Only a limited number of studies can be accessed from one database. Research limits can be expanded by using other databases. In addition, although a research directly related to CAU is attempted, the content and quality of publications may not be interpreted by bibliometric analysis. This may mean that some publications are included in the analyses even though they address a different topic than CAU.

CONCLUSION

The number of studies using bibliometric analysis as a tool in science research has been increasing in recent years. Because it facilitates the understanding of many complex researches in a field. This study was conducted using bibliometric analysis in order to create a resource that researchers can read while conducting research in the field of CAU. This study can provide detailed information about the situation in the current literature in the field of CAU. Analyses on authors, sources, keywords and countries that should be researched while reading about the field are included. It is thought that reading and analysing this study carefully in academic research will facilitate researchers to obtain information in the field of CAU.

Author Contributions

Research Design (CRediT 1) Author 1 (%50) - Author 2 (%30) – Author 3 (%20)

Data Collection (CRediT 2) Author 1 (%50) - Author 2 (%10) - Author 3 (%30) - Author 4 (%10)

Research - Data Analysis - Validation (CRediT 3-4-6-11) Author 1 (%50) - Author 2 (%30) - Author 3 (%15) - Author 4 (%5)

Writing the Article (CRediT 12-13) Author 1 (%50) - Author 2 (%30) - Author 3 (%15) - Author 4 (%5)

Revision and Improvement of the Text (CRediT 14) Author 1 (%50) - Author 2 (%30) - Author 3 (%15) - Author 4 (%5)

Finance

The study did not receive any financial support.

Conflict of Interest

There is no conflict of interest.

Sustainable Development Goals (SDG)

Does not support

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