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BACTERIAL APPLICATIONS FOR YIELD AND QUALITY INCREASE IN MELON PLANTS: AN ALTERNATIVE RESEARCH TO CHEMICAL FERTILIZER USE

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Abstract

In this study, the effects of bacterial strains on some yield and quality parameters of melon, a vegetable species cultivated worldwide and of significant economic value, were investigated under field conditions. Within the scope of the study, bacteria were isolated from different sources and the plant growth promoting mechanisms of the obtained strains were determined. The effects of bacterial strains on plant development were evaluated in a field experiment established according to a randomized complete block design with three replications, and the experiment was carried out repetitively for two years. The experiment included 11 different treatments: IT 22 (*Bacillus safensis*), IT 22 + fertilizer, IT 63 (*Acinetobacter calcoaceticus*), IT 63 + fertilizer, IT 93 (*Acinetobacter calcoaceticus*), IT 93 + fertilizer, IT 115 (*Serratia rubidaea*), IT 115 + fertilizer, Mix (IT 22 + IT 63 + IT 93 + IT 115), mix + fertilizer, and control. While the best result in terms of all parameters examined was obtained from the mix + fertilizer application, only the highest water-soluble dry matter amount (9.9%) was measured in the IT 93 + fertilizer application. Although the best results were obtained from the mix + fertilizer application, the results of the study showed that the fertilizer-free IT 93 and mix applications provided greater yield and quality increases in melon compared to the control with only fertilizer application, and as a result, the application of bacteria alone or in mixtures could be a good alternative to the use of chemical fertilizers in agricultural production. This result has been an indication that a significant reduction in chemical use will be achieved by including the determined bacterial strains in fertilization programs. This study will contribute to the development of new and efficient methods for the protection of producers and the environment by providing both economic and ecological benefits.

Key words: Biofertiliser, *Cucumis melo*, PGPB, Plant nutrition, Sustainable agriculture.

Introduction

Cucumis melo, which is cultivated in large areas worldwide and has the potential to increase the income of producers, is a garden plant in the Cucurbitaceae family (Makful *et al*., 2017). Consumption of melon, which contains abundant amounts of Mg, Ca, P, Fe and is one of the richest sources of Na, helps reduce the risk of heart diseases, degenerative diseases (depression, Alzheimer's, muscle and joint pain), and cancer (Lester, 2008). In 2021, global melon production reached 42 million tons, with the majority produced by China. With a production quantity of 1,638,638 tons, Türkiye ranked as one of the top melon-producing countries after China (Anon., 2021).

With population growth, the demand for agricultural products is increasing. Unfortunately, in order to meet this demand, chemicals are largely used to increase the yield and fruit quality of the plant in traditional agricultural systems where intensive inputs are used (Martínez *et al*., 2019). However, unconscious and excessive use of these chemicals in agricultural production causes pollution of natural resources such as soil and water, decrease in the diversity of microorganisms in the soil, and thus decrease in soil fertility and quality. This situation leads to a decrease in the quality and quantity of agricultural products (Doktor & Bulut, 2023). For this reason, in recent years, the development of sustainable production systems targeting the use of organic inputs in agriculture has become important. The use of biotechnological methods in this regard makes it possible to reduce chemical inputs and produce more and healthier food; therefore, studies in this field are increasing day by day (Bhardwaj *et al*., 2014). Sustainable or good agricultural practices aims to use natural resources such as soil, water, and plants effectively and efficiently, to protect the environment, to ensure food safety for public health and, finally, to leave a livable nature to future generations. One of the environmentally friendly alternative methods to be applied at this point is the use of plant growth-promoting bacteria (PGPB). This understanding, which is focused on sustainable agriculture, reveals the necessity of biological applications instead of chemical use in agricultural production (Toprak, 2012). Plant growth-promoting bacteries have a growth-promoting effect in plants through different means, such as increasing nutrient availability (phosphorus and potassium solubility, biological nitrogen fixation, siderophore production) and phytohormone production (Mia *et al*., 2012; Dönmez & Temel, 2023). It also increases plant resistance to biotic stress caused by various pathogens through some mechanisms (secretion of antagonistic substances, antibiotic production, etc.) (Khan *et al*., 2021). However, it has been reported that some Plant growth-promoting bacteries can help plants resist abiotic stresses, and at this point, their use in agriculture is seen as a new and promising approach to increase the success of remediating contaminated soils (Tak *et al*., 2012).

In various previous studies, it has been determined that bacteria in the genera *Bacillus, Pseudomonas, Serratia, Enterobacter,* and *Streptomyces* have great potential in vegetable cultivation, promote the development of many plant species, and increase yield (Bhattacharyya & Jha, 2012; Karpagam & Nagalaleshmi, 2014; Yıldırım *et al*., 2023; Dönmez *et al*., 2024). Therefore, the potential of bacteria to increase yield and quality in melon cultivation offers an important opportunity to develop new approaches in agricultural production. As far as is known from the

literature reviewed on the subject, it has been observed that no study has been conducted on the effect of bacterial applications on yield in Sürmeli F1 melon variety and that no bacteria were isolated and used for this purpose from some plants and volcanic rocks subject to isolation in the study. In this respect, it is very important to reveal how various bacterial species increase the development and product quality of the melon plant. Thus, by reducing the use of chemical fertilizers with bacterial applications, both the productivity of producers can be increased and the negative environmental effects of commonly used chemicals can be reduced while contributing to sustainable agriculture. Based on this, the aim of the current study was to evaluate the effects of bacterial applications on melon cultivation under field conditions. Within the scope of this purpose, bacteria were isolated from different sources, and their diagnoses were made based on fatty acid profiles and 16S rDNA sequence analysis. Various mechanisms of the obtained strains that play a role in increasing plant development were investigated, and the effects of bacterial strains applied alone and in combination on some yield and quality parameters of melons grown under field conditions were revealed.

Material and Methods

Test plant and bacterial strains used in the study: In order to determine the effect of bacterial strains on some yield and quality parameters of melon plants, the Sürmeli F_1 melon variety, which is the most preferred by producers in Iğdır, was used as plant material. The strains whose effects on plant development were investigated in the study were obtained as a result of isolation from samples taken from rhizosphere and phyllosphere regions of some alternative forage plants (*Chepodium qinoa, Amaranthus paniculatus, Atriplex nitens,* and *Salsola rutenica*) with the potential to grow in extreme conditions and from volcanic rocks collected from Taşburun Village in Iğdır.

Isolation of bacterial strains: The surface disinfected samples were cut into small pieces and kept in sterile water. Lines were planted from these suspensions on Nutrient Agar (NA) medium. The petri dishes were incubated at 27ºC and each of the bacterial colonies with different colors and characteristics that developed after incubation were purified. 10 g of soil from the rhizosphere region of the plant samples was weighed and placed in a sterile Erlenmeyer. 90 ml of sterile water was added and shaken on a shaker for 30 minutes. Then, a six-fold serial dilution of the suspension in the Erlenmeyer was prepared and 0.1 ml was taken from the last 3 dilutions and placed in the petri dishes and spread on the medium with a glass rod. The planted petri dishes were left in an incubator set at 27oC to develop and each of the bacterial colonies with different colors and shapes that developed after incubation were purified (Saygılı *et al*., 2006).

Soil properties of the experimental area: In order to determine whether the nutrients required by the plants were sufficient in the area where the experiment was established, soil samples were taken and mixed to represent the experiment area before planting and then analyzed at Igdir University, Research Laboratory Application Centre.

Identification of strains: Biochemical properties of isolated bacterial strains were determined by oxidase, catalase, gram reaction (Narayanasamy, 2001), starch hydrolysis (amylase production), and levan colony formation tests (Hélias *et al*., 2012). A hypersensitive reaction test was used to test whether the strains were pathogenic or not. At the same time, the strains were identified by fatty acid methyl ester analysis (Sasser, 1990) and 16S rDNA sequence analysis (service received from Ficus Biotechnology).

Determination of plant growth promoting properties of the bacterial strains in the study: Bacterial cultures grown on nutrient agar (NA) media for 24-48 hours were used in all tests conducted to determine the plant growth-promoting properties of bacterial strains. Nitrogen-fixing properties of bacterial strains were determined in N-free solid malate-sucrose medium. Bacterial growth observed in the medium was evaluated as a positive result (Cattelan *et al*., 1999). Potassium solubilization of bacterial strains was determined using Aleksandrov medium. The lightening of color (formation of a transparent area) around the colonies growing in the medium was accepted as positive for its potassium solubilization feature (Meena *et al*., 2014). Phosphorus solubilization of bacterial strains was tested in the National Botanical Research Institutes' Phosphate Growth Medium (NBRIP-BPB) liquid medium. The color change in the medium (color change from dark purple to light blue or becoming transparent) was considered a positive result (Nautiyal, 1999). Calcium solubilization of bacterial strains was determined using Yeast Extract Dextrose (YDC) medium. The formation of a transparent zone around the bacterial colonies was considered a positive result (Meena *et al*., 2014). The capacity of bacterial strains to produce 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme was tested in Dworkin and Foster (DF) Salt medium according to the procedure developed by Penrose & Glick (2003). Colony growth on the medium was considered ACC-deaminase positive. Bacteria were tested for siderophore production using Crom Azurol S (CAS) agar medium. The formation of orange-colored areas around the colonies growing in the petri dish was evaluated as positive siderophore production of the strains (Louden *et al*., 2011).

Determination of phytohormone production of bacterial strains included in the study: The hormone production of the bacterial strains tested for their effect on plant growth was determined qualitatively and quantitatively using an HPLC (high liquid pressure chromatography) device. Hormone extraction from bacterial strains was performed according to the method reported by Atıcı *et al*., (2003). The amount of hormones produced by the analyzed bacteria was calculated using the calibration graph method (y= 107.4x-58.5 for zeatin, y= 8.8x+5.5 for gibberellic acid, y= 44.7x+16.5 for indoleacetic acid, y=245.4x-65.8 for abscisic acid, and y=48.2x-160.5 for salicylic acid). The calibration graphs of the hormones are given in (Fig. 1).

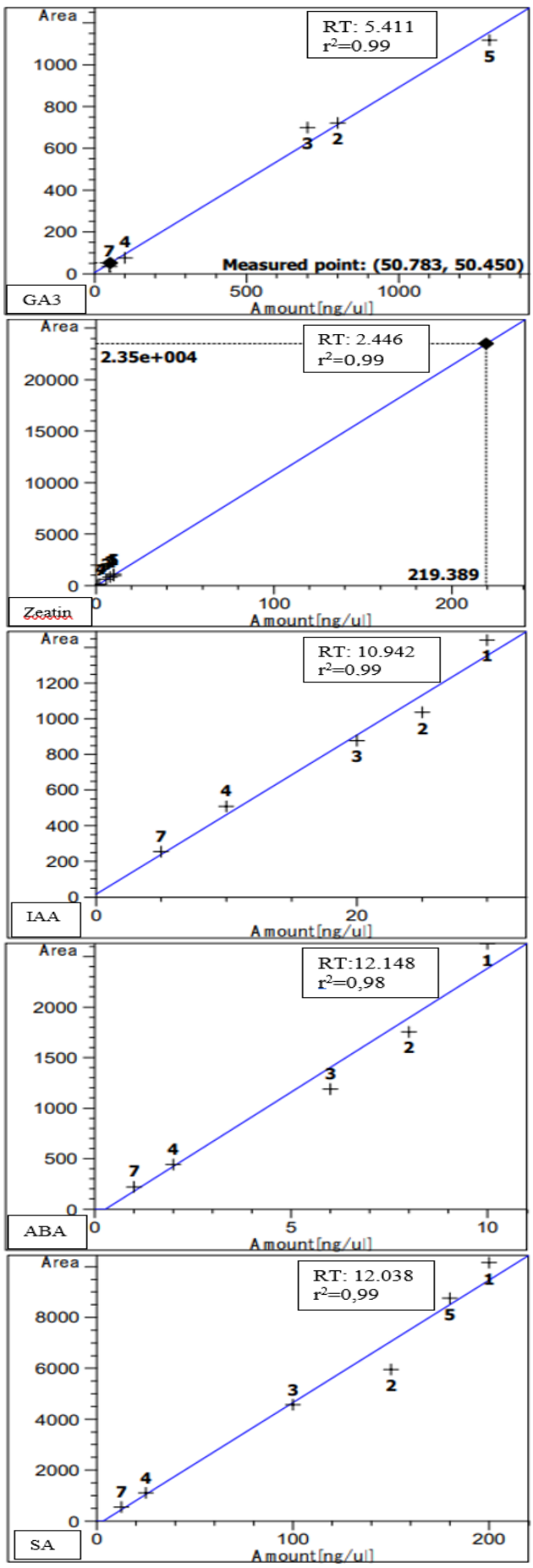

Fig. 1. Calibration graphs of the analyzed hormones.

The experiment was established at Iğdır University, Faculty of Agriculture, Agricultural Research and Application field (39°55'44"N 44°05'42"E) according to the Randomized Block Trial Design with three replications (Fig. 2) and carried out in Iğdır during the melon production seasons (March-August) of 2021 and 2022 with two replications. After the planting area was leveled by plowing with a crowbar, it was parceled with a distance of 70 cm above rows, 120 cm between rows, 140 cm between applications in the block, and 200 cm between blocks. There were 3 rows with 12 plants in each plot. Then, drip irrigation pipes were drawn in the experimental area. The study included 11 different applications (IT 22, IT 22 + fertilizer, IT 63, IT 63 + fertilizer, IT 93, IT93 + fertilizer, IT 115, IT 115 + fertilizer, Mix (IT 22 + IT 63 + IT 93 + IT 115), Mix + fertilizer, and only fertilizer). All cultural treatments were carried out regularly during the period of the experiment.

The vials used to grow the seedlings to be transplanted to the field were disinfected with 2% sodium hypochlorite and then filled with sterile soil and peat (1:1). After surface disinfection, the seeds were kept in the bacterial solutions prepared at a density of 10^6 cfu/ml for 2 hours and then dried. Then, one seed was planted in each vial hole when the plants had 4-5 leaves, 5 ml of bacterial solution per plant was applied to the soil from the plant root collar. 1 month after the seedlings were transferred to the field, 100 ml/plant of bacterial solution was applied to the plant roots (Fig. 3). In the applications where the effects of bacteria and fertilizer were tested together, half of the fertilizer adjusted to a total of 13,45 kg da-1 N and 8,7 kg da-1 P was applied before planting and the other half during the flowering period (Kokalis-Burelle *et al*., 2003). Ammonium sulfate was used as nitrogenous fertilizer, and triple super phosphate was used as phosphorus fertilizer. As a mixed application, equal volumes of solutions of 4 bacterial strains were taken, and the mixture was applied to the soil from the root collar of the plant. Only chemical fertilizer was given to the plants in the control group.

Yield and quality parameters examined in the study: Germination rates of seeds (%) were determined according to Anon., (1993), main stem length (cm) according to Kutsal (2017), plant fresh weight (g) according to Emrebaş (2010), leaf chlorophyll content (%) using the SPAD-502 device according to Rostami *et al*., (2008), fruit peel thickness (mm), fruit diameter (cm), fruit length (cm), fruit flesh thickness (mm), number of fruits per plant, and fruit yield per plant according to Karabulut (2018), average fruit weight, and water-soluble dry matter content (%) according to Eşiyok *et al*., (2005), and yield per decare (kg/da) according to Kutsal (2017). When the fruits reached harvest maturity, after the edge effect of the plots was removed, 3 plants representing the applied plots were selected and uprooted, and qualitative and quantitative measurements were made.

Data analysis

The significance levels of the variation sources of the data obtained in the study were determined using the SPSS (Anon., 2008) statistics program, and the significant means were compared using the Duncan test at $p \leq 0.01$. In addition, correlation analysis was performed to reveal the relationship between the parameters examined in the same statistics program.

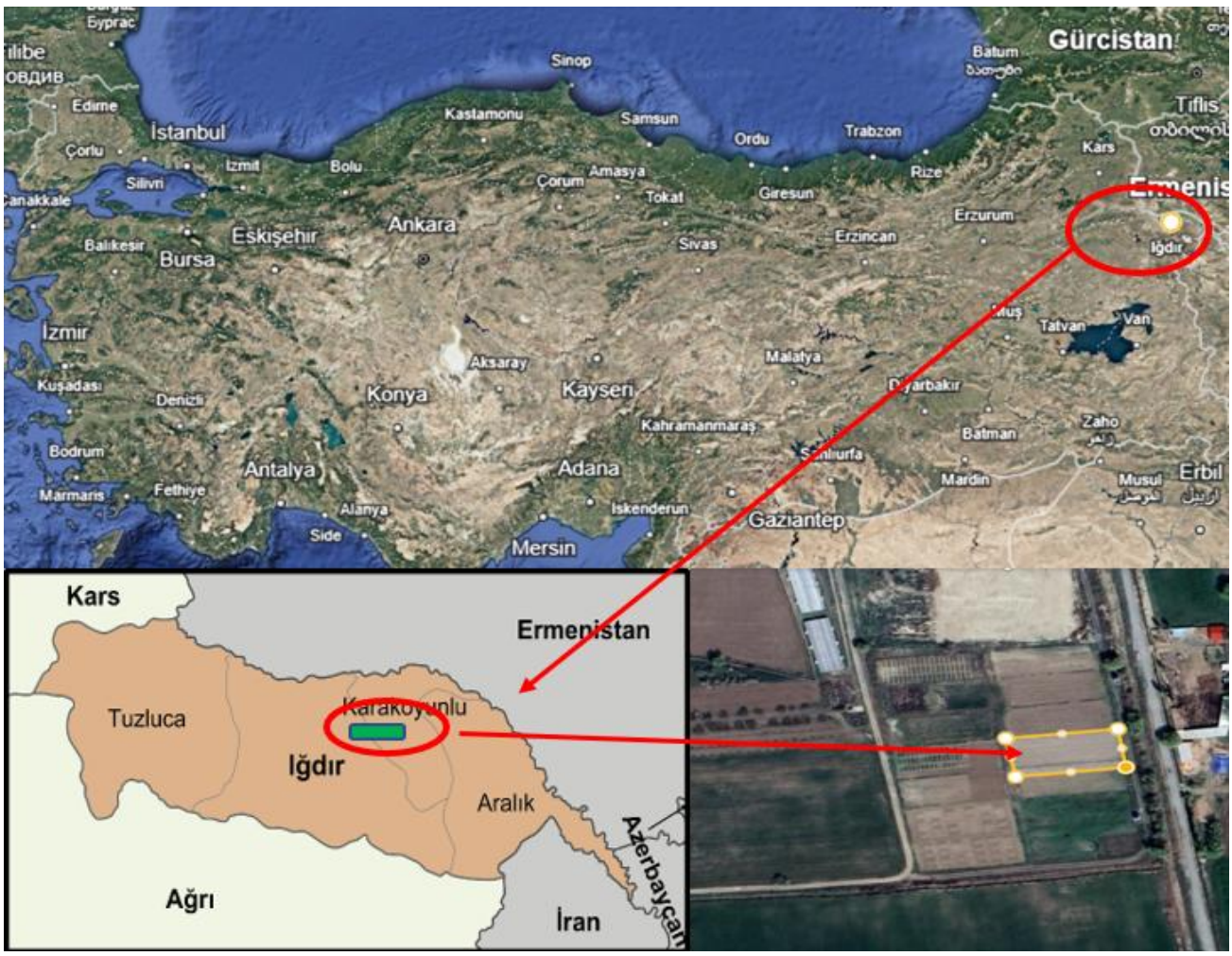

Fig. 2. Image of the experimental area where the effect of bacterial applications on melon yield was tested.

Fig. 3. Inoculation of melon seeds with bacteria, and sowing of melon seedlings in the field (below).

Results

As a result of the isolation performed in the study, 189 strains were obtained. All of the strains were identified by fatty acid methyl ester analysis and their biochemical properties were determined. Four strains thought to have the potential to increase plant growth were selected and used in the field trial. These four strains were also molecularly identified by 16S rDNA sequence analysis. Table 1 presents the identification results and biochemical properties of the strains based on their fatty acid profiles. The 16S rDNA sequence analysis results of the strains are given in Table 2.

When the values in Table 2 were examined, it was determined that after matching the 16S rDNA gene sequences of the strains with the accessions in GenBank as a result of the BLAST analysis, IT 22 of the strains matched with the *B. safensis* species, IT 63 and IT 93 strains matched with the *A. calcoaceticus* species, and IT 115 matched with the *S. rubidaea* species with a 99% identification percentage.

The effects of precipitation and temperature values of the period in which the experiment was conducted on the study results were evaluated, and the climate data for this period were obtained from the Iğdır Meteorology 16th Regional Directorate (Table 3). The soil analysis results of the trial area are presented in Table 4.

The results of plant growth promoting properties of bacterial strains such as nitrogen fixation, potassium and phosphorus solubilization, ACC-deaminase activity, phytohormone and siderophore production are given in Table 5. It was determined that all tested bacterial strains fixed nitrogen, solubilized phosphorus and potassium and produced siderophore. The highest zeatin (51,8 ng/µl) and siderophore production (23,0 mm) were detected in *Acinetobacter calcoaceticus* strain IT 93 (Table 5).

Table 1. Identification results and some characteristics of bacteria used as PGPB.

S. No.	MIS identification result	O*	K	GR	LKF	A	HR	Isolation material
IT 22	*Enterobacter hormaechei*	-	+	-	+	-	-	*Amaranthus paniculatus*- trunk
IT 63	*Acinetobacter calcoaceticus*	-	+	+	-	-	-	Volcanic rock
IT 93	*Acinetobacter calcoaceticus*	-	+	+	+	-	-	*Salsola rutenica*-root
IT 115	*Serratia rubidaea*	-	+	-	+	-	-	*Atriplex nitens*-soil

SN: Strain no, MIS: Microbial identification system, O: Oxidase, K: Catalase, GR: Gram reaction, LKO: Levan colony formation, A: Amylase, HR: Hypersensitive reaction

Table 2. Identification results of 16S rDNA sequence analysis of bacterial strains.

Strain No.	16S rDNA identification result	E value	Identification percentage	Access No.
IT 22	*Bacillus safensis*	0,0	99,61	MN365895.1
IT 63	*Acinetobacter calcoaceticus*	0.0	99,07	KX774370.1
IT 93	*Acinetobacter calcoaceticus*	0.0	99,51	OQ421667.1
IT 115	*Serratia rubidaea*	0,0	99,86	MT507224.1

Table 3. Climate data for the period when the experiment was conducted.

Months	Total rain fall (mm)			Average temperature (°C)			Average relative humidity (%)		
	2021	2022	LAG*	2021	2022	LAG	2021	2022	LAG
March	46,4	24,8	21,3	7,4	5,1	7,0	55,3	54,9	50,2
April	18,4	25,8	38,5	17,4	15,7	13,4	44,0	43,9	49,2
May	42,1	54,8	50,1	21,1	17,0	17,6	46,7	53,9	51,5
June	0,7	26,0	32,8	26,8	24,6	22,3	34,4	47,6	45,9
July	32,4	0,2	14,9	27,4	27,7	26,2	46,0	37,6	43,2
August	8,3	0,4	9,8	27,4	27,9	25,7	40,6	39,7	44,5
T./Ave.	148,3	132,0	167,3	21,3	19,7	18,7	44,5	46,3	47.4

*LAG: Long-Year Average (1978-2020), T: Total, Ave: Average

Table 4. Soil properties of the experimental area.

Saturation (%)	pH	Total salt (%)	Lime (%)	Organic matter (%)	Total nitrogen (%)	Phosphorus (P_2O_5) kg/da	Potassium (K_2O) kg/da
69,86	8,38	0,26	1,81	1,42	0,071	4,60	28,14
Clay-loamy	Slightly alkaline	Slightly saline	Calcareous	Less	Moderately	Less	Moderately

Table 5. Plant growth promoting properties of bacterial strains.

Strains	N	K	P	Ca	ACC	S	Phytohormone production (ng/µl)				
							GA	Z	ABA	IAA	SA
IT 22	+	+	+	-	+	21,0	-	16,5	-	-	-
IT 63	+	+	+	-	+	14,0	14,6	-	-	-	-
IT 93	+	+	+	-	+	23,0	13,3	51,8	-	-	-
IT 115	+	+	+	-	+	14,0	-	16,7	-	-	-

N: Nitrogen, K: Potassium, P: Phosphorus, Ca: Calcium, ACC: 1-aminocyclopropane-1-carboxylic acid-deaminase, S: Siderophore, GA: Giberallic acid, Z: Zeatin, IAA: Indole acetic acid, ABA: Abscisic acid, SA: Salicylic acid, +: Positive, -: Negative (IT 22: *Bacillus safensis*, IT 63: *Acinetobacter calcoaceticus*, IT 93: *Acinetobacter calcoaceticus*, IT 115: *Serratia rubidaea*)

It was determined that 100% germination was achieved in all seeds inoculated with bacterial strains. The analysis results of the effects of the applications on main stem length, plant fresh weight, and fruit diameter are presented in Table 6. According to the results of the statistical analysis, it was seen that the effects of year and bacterial application were significant on all the parameters examined, while the interaction of bacterial application and year was insignificant.

When the average values in Table 6 are examined, the highest main stem length in terms of applications was measured in plants in the mixture + fertilizer application. The lowest main stem lengths were measured in IT 115 and IT 22 applications, which are in the same statistical group. When evaluated in terms of years, it was determined that the plants in 2022 had higher main stem length compared to 2021. When the average values for plant fresh weight were examined, the highest values were obtained in mixture + fertilizer and IT 93 + fertilizer applications, respectively. It was seen that the lowest fresh plant weight was obtained from IT 115 application. When evaluated in terms of years, it was determined that the fresh plant weight in 2022 was higher than in 2021. When the applications were evaluated in terms of fruit diameter, the highest fruit diameter values were measured in the Mixture + Fertilizer application and the lowest in the control group. The fruit length, fruit flesh and fruit peel thickness values obtained as a result of the applications in the study carried out for two years are given in Table 7.

When Table 7 is examined, it is understood that the plants in the mixture + fertilizer application had the longest fruit length, while the plants in the control group were the shortest. When the results were evaluated on a yearly basis, it was determined that the fruit length values were higher in 2022 compared to 2021. While the highest fruit flesh thickness was measured in the mixture + fertilizer application, the lowest fruit flesh thickness was determined in IT 63 and control applications. On the other hand, fruit flesh thicknesses showed significant differences according to years, and the highest value was determined in 2022. Fruit peel thicknesses showed significant differences depending on the bacterial applications, and the best results were obtained from the mixture + fertilizer application (Table 6). When evaluated on a yearly basis, thinner fruit peel thickness was measured in 2022.The analysis results of the effects of the applications on the amount of water-soluble dry matter, the number of fruits per plant, and chlorophyll content in the leaf are given in Table 8.

Table 6. Effect of bacterial applications on main stem length, plant fresh weight and fruit diameter.

	Main stem length (cm)	Plant fresh weight (g)	Fruit diameter (cm)
		Applications	
IT115	233,0^{d**}	1164,9^{e**}	18,4a-^{c**}
IT115+Fertilizer	247,5^{b-d}	1345,7^{cd}	18,9^{ab}
IT22	238,9^{d}	1285,2^{d}	17,0^{cd}
IT22+Fertilizer	258,0^{a-d}	1436,7^{bc}	18,7^{a-c}
IT63	246,0^{b-d}	1351,3^{cd}	17,0^{cd}
IT63+Fertilizer	258,8^{a-d}	1422,6^{bc}	18,0^{bc}
IT93	255,3^{b-d}	1449,7^{b}	17,6^{bc}
IT93+Fertilizer	269,1^{a-c}	1566,5^{a}	18,5^{a-c}
Mix	272,0^{ab}	1461,5^{b}	17,3^{b-d}
Mix+Fertilizer	284,8^{a}	1461,5^{b}	19,9^{a}
Fertilizer	240,7^{cd}	1297,3^{d}	15,7^{d}
		Years	
2021	249,4^{b}	1355,6^{b}	17,1^{b}
2022	260,4^{a*}	1442,9^{a**}	18,7^{a**}

** %1, * %5. Data are averages of three replicates. The difference between values with the same letters in the same column is statistically insignificant (IT 22: *Bacillus safensis*, IT 63: *Acinetobacter calcoaceticus*, IT 93: *Acinetobacter calcoaceticus*, IT 115: *Serratia rubidaea*)

Table 7. Fruit length, fruit flesh and fruit shell thickness values obtained as a result of the applications.

	Fruit length (mm)	Flesh thickness (mm)	Shell thickness (mm)
		Applications	
IT115	22,3^{bc*}	34,3b^{c**}	11,0b^{c**}
IT115+Fertilizer	24,3^{ab}	36,5^{b}	10,3^{cd}
IT22	22,1^{bc}	34,8^{bc}	12,3^{a}
IT22+Fertilizer	23,7^{a-c}	36,5^{b}	11,4^{ab}
IT63	22,0^{bc}	33,5^{c}	12,4^{a}
IT63+Fertilizer	24,3^{ab}	35,2^{bc}	10,0^{cd}
IT93	22,3^{bc}	35,2^{bc}	11,9^{ab}
IT93+Fertilizer	22,8^{a-c}	35,6^{bc}	11,4^{ab}
Mix	23,5^{a-c}	36,3^{b}	10,9^{bc}
Mix+Fertilizer	24,7^{a}	38,7^{a}	9,4^{d}
Fertilizer	21,6^{d}	33,7^{c}	11,0^{bc}
		Years	
2021	22,4^{b}	34,7^{b}	11,5^{a}
2022	23,7^{a**}	36,3^{a**}	10,7^{b**}

** %1, * %5. Data are averages of three replicates. The difference between values with the same letters in the same column is statistically insignificant. (IT 22: *Bacillus safensis*, IT 63: *Acinetobacter calcoaceticus*, IT 93: *Acinetobacter calcoaceticus*, IT 115: *Serratia rubidaea*)

Table 8. Water-soluble dry matter, leaf chlorophyll content and the number of fruits per plant obtained as a result of the applications.

	Water-soluble dry matter (%)	Leaf chlorophyll content (%)	Number of fruits per plant (number/plant)
		Applications	
IT115	8,6$^{b\text{-}d**}$	69,1^{ef**}	2,34^{d**}
IT115+Fertilizer	9,0^{bc}	72,5$^{d\text{-}f}$	2,43^{cd}
IT22	8,6$^{b\text{-}d}$	70,9$^{d\text{-}f}$	2,31^{d}
IT22+Fertilizer	9,3^{ab}	76,0$^{c\text{-}e}$	2,59^{b}
IT63	8,0^{de}	80,3$^{b\text{-}d}$	2,57^{b}
IT63+Fertilizer	9,0^{bc}	82,2^{bc}	2,64^{ab}
IT93	9,3^{ab}	79,5$^{b\text{-}d}$	2,62^{ab}
IT93+Fertilizer	9,9^{a}	86,1^{b}	2,67^{ab}
Mix	8,3$^{c\text{-}e}$	88,9^{ab}	2,70^{ab}
Mix+Fertilizer	8,8$^{b\text{-}d}$	96,8^{a}	2,77^{a}
Fertilizer	7,6^{e}	64,3^{f}	2,55^{bc}
		Years	
2021	8,2^{b}	76,2^{b}	2,48^{b}
2022	9,3^{a**}	81,4^{a**}	2,64^{a*}

** %1, * %5. Data are averages of three replicates. The difference between values with the same letters in the same column is statistically insignificant. (IT 22: *Bacillus safensis*, IT 63: *Acinetobacter calcoaceticus*, IT 93: *Acinetobacter calcoaceticus*, IT 115: *Serratia rubidaea*)

Table 9. Effect of bacterial applications on average fruit weight and melon yield.

	Average fruit weight (g/fruit)	Fruit yield per plant (g/plant)	Fruit yield per decare (kg/da)
		Applications	
IT115	2461.5^{e**}	5757.0^{h**}	4835.8^{h**}
IT115+Fertilizer	2727.5^{cd}	6628.3^{fg}	5567.8^{fg}
IT22	2538,5^{de}	5875.3^{h}	4935.3^{h}
IT22+Fertilizer	3067.5^{b}	7924.7^{bc}	6656.8^{bc}
IT63	2432.3^{e}	6226.1^{gh}	5230.0^{gh}
IT63+Fertilizer	2749.3^{cd}	7262.4^{de}	6100.4^{de}
IT93	2930.8^{bc}	7688.1^{cd}	6458.0^{cd}
IT93+Fertilizer	3018.8^{b}	8050.0^{bc}	6762.0^{bc}
Mix	3074.2^{b}	8295.2^{b}	6968.0^{b}
Mix+Fertilizer	3307.5^{a}	9146.2^{a}	7682.8^{a}
Fertilizer	2730.8^{cd}	6957.6^{ef}	5844.4^{ef}
		Years	
2021	2748,3^{b}	6843,3^{b}	5748,4^{b}
2022	2895,1^{a**}	7667,8^{a**}	6440,9^{a**}

** %1, * %5. Data are averages of three replicates. The difference between values with the same letters in the same column is statistically insignificant. (IT 22: *Bacillus safensis*, IT 63: *Acinetobacter calcoaceticus*, IT 93: *Acinetobacter calcoaceticus*, IT 115: *Serratia rubidaea*)

Table 10. Correlation coefficients and significance levels of yield and quality parameters.

	AFW	NFPP	FYPP	FYPD	FT	FL	ST	FD	WSDM	LCC	PFW	MSL
AFW	1.00	0.23$^{n.s.}$	0.90**	0.90**	0.50**	0.36**	-.22$^{n.s.}$	0.38**	0.48**	0.29*	0.52**	0.35**
NFPP		1.00	0.62**	0.62**	0.24*	0.23$^{n.s.}$	-.15$^{n.s.}$	0.24$^{n.s.}$	0.24$^{n.s.}$	0.42**	0.32**	0.25*
FYPP			1.00	1.00**	0.52**	0.39**	-0.24*	0.42**	0.49**	0.43**	0.56**	0.39**
FYPD				1.00	0.52**	0.39**	-0.24*	0.42**	0.49**	0.43**	0.56**	0.39**
FT					1.00	0.38**	-.20$^{n.s.}$	0.39**	0.17$^{n.s.}$	0.24$^{n.s.}$	0.50**	0.22$^{n.s.}$
FL						1.00	-0.44**	0.35**	0.15$^{n.s.}$	0.50**	0.41**	0.54**
ST							1.00	-0.35**	-0.08$^{n.s.}$	-0.19$^{n.s.}$	-0.15$^{n.s.}$	-.15$^{n.s.}$
FD								1.00	0.38**	0.52**	0.57**	0.14$^{n.s.}$
WSDM									1.00	0.22$^{n.s.}$	0.49**	0.18$^{n.s.}$
LCC										1.00	0.67**	0.42**
PFW											1.00	0.34**
MSL												1.00

** %1, * %5, n.s.: Non-significant. FYPP; Fruit yield per plant, AFW; Average fruit weight, NFPP: Number of fruits per plant, FYPD; Fruit yield per decare, FT: Flesh thickness, FL: Fruit length, ST: Shell thickness, FD: Fruit diameter, WSDM: Water-soluble dry matter, LCC: Leaf chlorophyll content, PFW: Plant fresh weight, MSL: Main stem length

The highest amount of water-soluble dry matter was obtained with Acinetobacter calcoaceticus 93+Fertilizer and the lowest amount of water-soluble dry matter was obtained as a result of control applications, respectively. When the amounts of soluble dry matter by year were evaluated, it was seen that this value was higher in 2022. Leaf chlorophyll content showed significant differences depending on bacterial applications, and the highest chlorophyll content was found in plants applied with mixture + fertilizer. When the number of fruits per plant was examined in terms of bacterial applications, the highest number of fruits was determined in the mixture + fertilizer application. It was observed that the number of fruits per plant was higher in 2022 (Table 8). The average fruit weight, per plant and per decare, and fruit yield values obtained as a result of the practices and the averages of these values are presented in Table 9.

When Table 9 is examined, higher average fruit weight was obtained in 2022 compared to 2021. The highest fruit yield per plant and the highest fruit yield per decare were determined in the mixture + fertilizer application. In order to test whether there is a relationship between the yield parameters examined in the current study and to reveal the degree of the existing relationship, the correlation coefficients of the examined parameters were calculated, and the significance levels are presented in Table 10.

Table 10 shows that there is a very significant and positive relationship between average fruit weight and fruit yield per plant and per decare, and a very significant and moderately positive relationship between fruit flesh thickness, fruit length, fruit diameter, water-soluble dry matter, plant fresh weight, and main stem length. There was a very significant and positive relationship between the number of fruits per plant and fruit yield per plant and fruit yield per decare, and a very significant but moderately positive relationship between the number of fruits per plant and leaf chlorophyll content and plant fresh weight. While a very significant and quite high relationship was observed between fruit yield per plant and fruit yield per decare, a very significant and moderately positive relationship was found between fruit yield per plant and fruit flesh thickness, fruit length, fruit diameter, water-soluble dry matter content, leaf chlorophyll content, plant fresh weight, and main stem length. There was a very significant and moderately positive relationship between fruit yield per decare and fruit flesh thickness, fruit length, fruit diameter, water-soluble dry matter content, leaf chlorophyll content, plant fresh weight and main stem length. It was observed that there was a very significant and moderately positive relationship between fruit flesh thickness and fruit length, fruit diameter and plant fresh weight. There was a very significant and moderately inverse relationship between fruit length and fruit skin thickness and a very significant and moderately positive relationship between fruit length and fruit diameter, leaf chlorophyll content, plant fresh weight and main stem length. There was a very significant and moderately negative relationship between fruit length and fruit skin thickness. A very significant and moderately negative relationship was found between fruit skin thickness and fruit diameter. There was a very significant but moderately positive relationship between fruit diameter and water soluble dry matter content, leaf chlorophyll content and plant fresh weight. A very significant and moderately positive relationship was found between water-soluble dry matter content and plant fresh weight. There was a very significant and moderately positive relationship between leaf chlorophyll content and plant fresh weight and main stem length. There was a very significant and moderate positive relationship between plant fresh weight and main stem length.

Discussion

The use of bacterial strains in the nutrient cycle as biofertilizers provides a very important advantage for minimizing the negative effects caused by commercial fertilizers in agricultural production and for sustainable agriculture (Öztekin *et al*., 2015). For this reason, in the current study, the effect of bacterial strains on melon cultivation under field conditions was investigated, and it was found that mix + fertilizer application increased fruit yield per decare by 32% compared to the control group with only fertilizer application. This result showed that bacterial strains also increased the effectiveness of the applied fertilizer. The most desired thing in agricultural production is to increase productivity by making maximum use of the applied fertilizer. In different studies, it has been determined that bacterial strains increase plant growth by producing siderophores and phytohormones, fixing nitrogen, solubilizing phosphorus and potassium, and synthesizing stress-relieving enzymes. Additionally, it has been determined that some bacteria improve root development by increasing the accessibility of essential nutrients and support plant development by reducing the damage caused by stress by activating plant defense systems (Franche *et al*., 2009; Alves *et al*., 2015). Studies have shown that nitrogen, one of the most vital elements in plant growth and various metabolic activities, is converted by both symbiotic and free-living microorganisms into a form that plants can use by the nitrogenase complex (Dixon & Kahn, 2004; Ali *et al*., 2017), and that the structural and nitrogenase complex consists of regulatory genes involved in the biosynthesis of Fe protein and Fe-molybdenum cofactor activation (Parray *et al*., 2016). Additionally, some nitrogen-fixing rhizobacteria species have been found to have the ability to attenuate the increase in ethylene levels by synthesizing rhizobiotoxin, which inhibits the function of the ACC synthase enzyme (Vejan *et al*., 2016; Gouda *et al*., 2018). The mineralization of phosphorus, which has important roles in various plant metabolic processes such as photosynthesis and energy transfer (Sharma *et al*., 2013), by microorganisms has been found to increase the availability of phosphorus in soil, and mineralization is highly dependent on soil pH. Such microorganisms have been found to alter soil pH by secreting various organic and inorganic acids (malic, malonic, fumaric, oxalic, acetic, tartaric, glutamic, propionic, butyric, lactic, 2-keto gluconic, gluconic, and glyconic acids) through a mechanism known as rhizosphere acidification (Kumar *et al*., 2018; Umar *et al*., 2020). It is known that potassium, which plays a key role in the processes related to growth and development in plants, plays a role in activating more than 80 enzymes

involved in various energy metabolic processes (Adams & Shin, 2014; Wang *et al*., 2000). Potassium-solubilizing bacteria have been found to produce low molecular weight organic acids, and to release potassium from fixed potassium-containing minerals (Liu *et al*., 2012; Prajapati *et al*., 2013; Saiyad *et al*., 2015). It has been stated that the presence of iron in plants, which is essential for the survival of all organisms, is associated with the production of low molecular weight siderophores produced by bacteria. It has been determined that siderophores, reduce the population of pathogenic microorganisms, and increase plant growth by creating iron competition in the rhizospheric region (Saha *et al*., 2016; Hakim *et al*., 2021). Considering that all of the strains tested in this study have the ability to fix nitrogen, dissolve phosphorus and potassium, produce siderophores, and change soil pH with the acids they secrete, it can be stated that these properties play a role in increasing the development of the melon plant. In addition, bacterial strains have been found to help modulate hormone concentrations such as auxins (indoleacetic acid) and cytokinins in plants. Another important mechanism by which plant growth is promoted through bacterial strains is the reduction of plant ethylene levels, a hormone produced by plants under stress conditions (Gamalero & Glick, 2015). It has been shown that ACC (1-aminocyclopropane-1-carboxylate) produced by plant growth promotion bacteria is hydrolyzed by the 1-aminocyclopropane-1-carboxylate deaminase enzyme as a direct precursor of ethylene hormone in plants (Orozco-Mosqueda *et al*., 2020). The bacterial ACC deaminase enzyme has been found to function by degrading the ACC molecule into α-ketobutyrate and ammonia, thereby preventing the increase in ethylene production under various stress conditions, thus promoting the growth of plants and facilitating their survival (Santoyo *et al*., 2020). The fact that all four strains tested in this study showed positive results for ACC deaminase shows that they are important in supporting plant development.

In the current study, it was determined that seed coating with bacteria had a positive effect on seed germination and increased the germination rate compared to the control. Johnson *et al*., (2011) reported that strains in the cytokinin group that produced zeatin and also gibberellic acid had a positive effect on germination in melon seeds. It has been reported that this is due to the fact that plant growth promotion bacteries promote the synthesis of growth regulatory substances such as gibberellins, which trigger the activity of germination-specific enzymes such as α-amylase, proteases, and nucleases. Furthermore, indol acetic acid produced by bacteria is known to provide an ecological advantage to bacteria by promoting environmental adaptation under stress conditions such as UV, salt, and acidity (Bianco *et al*., 2006). Current study findings were evaluated, zeatin production was detected in 3 of the 4 strains tested for their effect on yield in melon and giberallic acid production was detected in 2. This result was found to be compatible with the findings of Kumar *et al*., (2014) and Vishwas *et al*., (2017), which reported that plant growth promotion bacteries significantly affected germination. In a study conducted with *Bacillus, Pseudomonas, Azotobacter* and *Acinetobacter* species, it was found that bacterial applications increased the main stem length and number of fruits per plant in bitter melon compared to the control (Singh & Jha, 2017). It has been reported that 9700 kg da-1 fruit yield was obtained as a result of root applications with bacterial strains in melon plants. It was also reported that the strains increased the shoot fresh weight (1186,2 g), root fresh weight (128,6 g) and main stem length (482,2 cm) of melon grown under greenhouse conditions compared to the control (Abraham Juárez *et al*., 2018). In the current study, it was determined that bacterial applications increased the number of fruits per plant compared to the control and the highest main stem length (284,8 cm) and fruit yield per decare (7682,8 kg) were obtained in the mix + fertilizer application. In different studies, it has been reported that applications with bacterial strains belonging to the *Pseudomonas, Serratia* and *Bacillus* genera significantly increased plant fresh weight, main stem length, number of fruits per plant, fruit diameter, average fruit weight and total fruit yield compared to the control (Almaghrabi *et al*., 2013; Yıldırım *et al*., 2022; Dönmez *et al*., 2024). In the present study, it was determined that the peel thickness varied between 9.4-12.4 mm whileIn another study in which the effect of bacterial applications on melon yield was tested, fruit yield of 2000-2300 kg/ha and fruit peel thickness values ranging between 13,58-14,56 mm were obtained (Kutsal, 2017). It is seen that the melon variety, the difference in bacterial strains and the region (different climate and soil characteristics) are effective in the values of the obtained parameters. It was determined that all applications in the study increased the chlorophyll content in the leaf compared to the control and the highest chlorophyll content (96,8%) was in the plants in the mix + fertilizer application. It has been reported by Kaçar, (2015) that there is a positive relationship between chlorophyll content and yield and that the increase in chlorophyll content increases plant yield. As stated in the current study, the highest yield per decare was obtained as a result of the mix + fertilizer application in which the highest chlorophyll content was measured, and the findings are parallel in this sense.

In this study, it was clearly observed that the most successful results were obtained with the combination of mix and mix + fertilizer among the applications. It has been reported that the combined application of strains with different mechanisms of action can be more effective than their single applications (Spaepen *et al*., 2007). This finding emphasizes the synergistic effect of bacterial applications in combination and paves the way for more efficient results. It has also been reported by Santhanam *et al*., (2019) that the use of bacterial strains in combination provides effectiveness in different environmental conditions by using more than one mechanism of action, that inconsistencies arising from the use of strains individually are overcome, and that the success of strains increases by stimulating the production of various enzymes and metabolites. This approach was found to be consistent with the results of the current study. It has also been reported that the effect of bacteria on plant development varies depending on the tested plant species, inoculation method, and inoculum density (Mangmang *et al*., 2015).

It is stated that genotype is primarily effective in increasing yield and quality; however, environmental factors such as temperature, light, humidity and CO_2 also play an important role (Özkaraman, 2023). It is reported that dry matter increases with increased photosynthesis, and the increased dry matter accumulates in the stem and storage organs, causing an increase in stem diameter. Studies on this subject indicate that dry matter accumulation in plants varies according to temperature, technical and cultural processes and affects the distribution of the produced dry matter to the organs of the plant (Uzun *et al.*, 1998; Sarıbaş *et al.*, 2018). When the temperature values for the period in which the experiment was conducted in the current study were examined, higher temperatures were observed in 2021 compared to 2022, especially during the flowering period of the plants. It has been reported that an increase in temperature increases vegetative development, but causes both a decrease in the formation of female flowers and a later onset of flowering, which reduces generative development and thus leads to a decrease in yield (Özkaraman, 2004). In this context, it was seen that the results of the current study were parallel and when the results obtained were evaluated, higher values were obtained in terms of the parameters examined, especially melon yield, in the second year of the study. The biocontrol effect of the successful strains can be evaluated in terms of pathogens causing diseases in other cucurbit plants. In addition, their effectiveness in controlling disease agents in pathosystems with different plant x environment x pathogen interactions can be tested.

Conclusions

Recently, chemical fertilizers used unconsciously in agricultural areas to increase yield and quality have caused a decrease in soil fertility and environmental pollution and have threatened the food security of the world population. In reducing the use of chemical fertilizers, which have high cost and pollution effects, the application of bacterial strains in a mixture will help to protect environmental health and maintain soil fertility. When the results are evaluated in terms of melon development, it is seen that the highest values are obtained from mix + fertilizer and mix applications. These results show that bacterial applications can be used as an important alternative in reducing the use of chemical fertilizers. The application of these strains will increase the effectiveness of the applied fertilizers, increase the availability of usable nutrients and reduce the application of chemical fertilizers, thus ensuring the sustainable use of natural resources and the improvement of plant production.

There are of course some difficulties in using bacteria as biofertilizers in agriculture. For example; conditions such as temperature, humidity and soil pH may reduce the effectiveness of bacteria or the benefits provided by bacteria may decrease over time. However, this problem can be prevented by selecting bacterial species with high adaptability (such as Bacillus, Pseudomona). It may bring additional costs in terms of cultivation, but it is seen as more advantageous financially when compared to the costs arising from the use of chemical fertilizers.

In addition to these effects, as is known, greenhouse gas emissions are also released during the production and use of chemical fertilizers. Integrating biofertilizer methods into agriculture will contribute to reducing the carbon footprint and therefore combating climate change.

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