Journal of Agricultural Faculty of Gaziosmanpasa University
Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/gopzfd

JAFAG (2025) 42(1), 21-26
ISSN: 1300-2910
E-ISSN: 2147-8848
DOI: 10.55507/gopzfd.1589102

Research Article

The effect of some beneficial bacteria on the yield of pepper plants

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Received: 21.11.2024 Accepted: 25.02.2025

Abstract: This study investigated plant growth-promoting characteristics of 389 candidate bacterial isolates collected during surveys conducted in 2017 by using nitrogen fixation, phosphorus reduction, and antagonistic activities tests. Six candidate bacterial isolates were identified as a result of the tests. The identification of these species was carried out using the MALDI-TOF MS technique, which identified species as *Enterobacter cloacae*, *E. aerogenes, Bacillus cereus*, *Microbacterium testaceum*, *Pseudomonas putida*, and *Acinetobacter calcoaceticus*. Pot and field trials were conducted using different combinations of these isolates. All isolates were used in the pot experiment, whereas isolates with the highest antagonistic activity and their combinations were used in the field trials. The pot trials were conducted using a randomized block design with five replications, while the field trials were conducted using a randomized complete block design with ten replications. The effects of these isolates on the yield of healthy pepper plants were investigated. Data relating to plant height, root length, stem diameter, number of fruits, plant fresh and dry weight, and root fresh and dry weight were collected. Results of the pot experiment revealed that combinations of *B. cereus* + *P. putida* and *P. putida* + *A. calcoaceticus* resulted in 15% higher yield compared to the control. Similarly, field trials indicated that *P. putida* improved the values of investigated traits by 12.3-37.2%. *Pseudomonas putida* is considered beneficial for future research based on the results of this study.

Keywords: PGPR, yield, bacteria, pepper

Bazı faydalı bakterilerin biber bitkilerinin verimi üzerindeki etkisi

Öz: Bu çalışmada, 2017 yılında yapılan sürveyler sonucunda toplanan 389 aday bakteri izolatının bitki büyümesini teşvik edici özellikleri araştırılmıştır. Elde edilen izolatların bitki gelişimini teşvik etme özelliklerini belirlemek amacıyla azotu bağlama, fosforu indirgeme ve antagonistik etki testleri yapılmıştır. Testler sonucunda altı adet aday bakteri izolatı belirlenmiştir. Bu türler MALDİ-TOF MS tekniği ile *Enterobacter cloacae, Enterobacter aerogenes, Bacillus cereus, Microbacterium testaceum*, *Pseudomonas putida* ve *Acinetobacter calcoaceticus* bakterileri olduğu tanılanmıştır. Saksı ve tarla denemeleri, bu izolatların farklı kombinasyonları kullanılarak yürütülmüştür. Saksı denemesinde tüm izolatlar kullanılırken, tarla denemesinde en yüksek antagonistik etkiye sahip izolatlar ve bunların kombinasyonları kullanılmıştır. Saksı denemeleri tesadüf parselleri deneme desenine göre 5 tekerrür olarak yürütülmüştür. Tarla denemeleri ise tesadüf blokları deneme desenine göre 10 tekerrürlü yürütülmüştür. Bu izolatların sağlıklı biber bitkisinin verim değerlerine etkileri araştırılmıştır. Saksı çalışması ve devamında yürütülen tarla çalışmasında bitki boyu, kök uzunluğu, gövde çapı, meyve sayısı, bitki yaş ağırlığı, bitki kuru ağırlığı, kök yaş ağırlığı ve kök kuru ağırlığı gibi parametreler ölçülmüştür. Sonuç olarak sera denemelerinde *B. cereus* + *P. putida* ile *P. putida* + *A. calcoaceticus* bakterilerin ikili kombinasyon uygulamalarında kontrole göre %15 daha fazla verim elde edilmiştir. Tarla uygulamalarında ise *P. putida* uygulaması değerlendirmeye alınan bitki parametrelerinde %12,3-37,2 arasında değişen oranlarda artışı sağlamıştır. Çalışma sonuçlarına göre özellikle *Pseudomonas putida* ile ileriye dönük çalışmaların yapılmasının yararlı olacağı düşünülmektedir.

Anahtar kelimeler: PGPR, verim, bakteri, biber

To cite this article: Kayaaslan, Z., Belgüzar, S., Yanar, Y., & Mirik, M. (2025). The effect of some beneficial bacteria on the yield of pepper plants. *Journal of Agricultural Faculty of Gaziosmanpasa University*, 42(1), 21-26. https://doi.org/10.55507/gopzfd.1589102

1. Introduction

Antagonistic bacterial isolates that promote plant growth not only activate the defense mechanisms of plants, but also stimulate seed germination, enhance root development, and improve nutrient uptake. Furthermore, they contribute to the plant's resistance to pathogens by producing hormones (Siddiqui, 2005). Different PGPR (plant growth-promoting Rhizobacteria) isolates improve yield and stress tolerance in plants while suppressing soil-borne pathogens (Kloepper et al., 1991). Endophytic bacteria have a high colonization ability in plant roots, leading to reduced stress and increased root development (Hardoim et al., 2008). Isolates belonging to genera Bacillus, Pseudomonas, and Agrobacterium are prominent in biological control as microorganisms associated with soil and plants (Fira et al., 2018). Considering all these properties, the use of PGPR is becoming increasingly important. Kang et al. (2007) selected 23 endophytic bacterial isolates among 150 isolates collected from pepper fields in Chungcheong and Gyeongsang provinces of Korea. These isolates were reported to increase root fresh weight by 73.9% and 41.5%, respectively, by stimulating systemic resistance in the plants. Mirik et al. (2008) isolated 3 Bacillus isolates from soil samples in the rhizosphere of pepper plants, then applied them alone or in combination to the pepper plants. Conequently, their application increased stem diameter, root length, root dry weight, shoot dry weight, and yield by 7-20%, 7-17%, 4.5-23.5%, 16.5-38.5%, and 11-33%, respectively.

This study isolated PGPRs from leaves, stems, root hairs, and rhizosphere regions of healthy pepper plants growing in Tokat Province. Six candidate isolates that could potentially promote plant growth were identified. These isolates identified by MALDI-TOF were *Enterobacter cloacae*, *E. aerogenes*, *Bacillus cereus, Microbacterium testaceum, Pseudomonas putida*, and *Acinetobacter calcoaceticus*, which were tested for their effects on pepper plant development under both greenhouse and field conditions in single, double, and triple combinations.

2. Materials and Methods

2.1. Materials

Samples were taken from 17 fields (7 from Tokat Center and 10 from Erbaa district) during September 2017 to obtain bacteria that promote plant growth. A total 389 candidate isolates were obtained by isolating the soil and capillary roots, stem and leaf parts of the pepper plant with different methods. These candidate isolates were subjected to the phosphate reduction test and 94 isolates with the best zone diameter were recorded. Nitrogen fixation ability test identified 38 candidate isolates (Kayaaslan 2021). Proteomic identification of the isolates determined by tobacco hypersensitivity, potato softening test and antagonistic effect tests were performed by Mustafa Kemal University, Plant Health Clinic Application and Research Center using MALDI-TOF MS technique. The baterial isolates tested were *Enterobacter cloacae, Enterobacter aerogenes, Bacillus cereus, Microbacterium testaceum, Pseudomonas putida*, and *Acinetobacter calcoaceticus*. The 'İstek F1' pepper variety was used in the experiments. Nutrient agar (NA) were used for the development of bacterial isolates.

2.2. Methods

The pot experiment was conducted in the greenhouses of the Research and Application Center at Tokat Gaziosmanpaşa University, while the field study was carried out in the field plots of the same center. Field trials were conducted with the three isolates that gave the best results in the pot experiment (ZE-7, ZE-12, and ZE-13).

2.3. Pot experiments

Pepper seedlings of 'İstek F1' variety in three true leaf stage were used in the pot experiments. The experiment was designed according to a randomized block design with five replications. Candidate bacteria were growen on NA medium at 25°C for 24-48 hours. The treatments are given in Table 1. The roots of the pepper seedlings were immersed in bacterial suspensions ($1x10^6$ cfu/ml) for 1 hour to ensure bacterial colonization. Afterwards, the seedlings were planted in 5-liter plastic pots filled with a soil:peat (1:1:1) mixture, with one plant per pot. The same bacterial suspensions were applied again 10 days after planting (50 ml per plant) to the root region of the plants (Belgüzar et al., 2021). Weekly observations were recorded following the second application throughout the growing season, and the number of fruits and total fruit weight were recorded for each plant. The aboveground and underground parts of the plants were separately collected in polyethylene bags and brought to the laboratory at the end of the production season. Plant height, root length, stem diameter, number of fruits, plant fresh and dry weight, and root fresh and dry weight were recorded.

Table 1. Treatments used in the pot experiments

Treatments	Bacterial isolates
NC	Distilled water (negative control)
ZE-2	*Enterobacter cloacae*
ZE-5	*Enterobacter aerogenes*
ZE-7	*Bacillus cereus*
ZE-8	*Microbacterium testaceum*
ZE-12	*Pseudomonas putida*
ZE-13	*Acinetobacter calcoaceticus*
ZE-7+2	*B. cereus + P. putida*
ZE-7+13	*B. cereus + A. calcoaceticus*
ZE-12+13	*P. putida + A. calcoaceticus*
ZE-7+12+13	*B. cereus + P. putida + A. calcoaceticus*

Table 2. Treatments used in the field experiments

Treatments	Bacterial isolates
NC	Distilled water treatment (negative control)
ZE-7	*Bacillus cereus*
ZE-12	*Pseudomonas putida*
ZE-13	*Acinetobacter calcoaceticus*
ZE-7+12	*B. cereus +P. putida*
ZE-7+13	*B. cereus +A. calcoaceticus*
ZE-12+13	*P. putida + A. calcoaceticus*
ZE-7+12+13	*B. cereus + P. putida+A. calcoaceticus*

2.4. Field trials

The field trials were conducted using three isolates (ZE-7, ZE-12, and ZE-13) that gave the best results in the pots experiments (Table 2). The seedlings of 'İstek F1' pepper variety were used in the field trial. The prepared bacterial suspensions were used to immerse the pepper seedlings roots for 1 hour to ensure bacterial colonization. Afterwards, the seedlings were planted in the field. Ten days after planting, a second bacterial suspension applications were made to the root region of the plants, 50 ml per plant. Plants roots were immersed in sterile distilled water as negative control. The study was set up according to randomized block design with 10 replications. Weekly observations were made throughout the growing season following the second application and the number of fruits and total fruit weight were recorded for each plant. The plants were harvested at the end of the production season and their above- and below-ground were collected separately in polyethylene bags and brought to the laboratory. Measurements for plant height, root length, stem diameter, number of fruits, plant fresh weight, and root fresh weight were taken. Additionally, above- and below-ground parts of the plants were dried, and their dry weights were recorded.

2.5. Statistical analyses

The differences between the applications were compared with ANOVA and Tukey multiple comparison test ($p \leq 0.05$).

3. Results and Discussion

3.1. Pots experiments

The effects of candidate bacterial isolates on plant height, root length, stem diameter, fruit number, plant fresh weight, plant dry weight, root fresh weight, and root dry weight in pepper are presented in Table 3. All treatments increased plant development to varying degrees, but this increase was statistically non-significant. The highest effect on plant height (15.2% increase compared with control) was observed for ZE-7+12 (*Bacillus cereus* + *Pseudomonas putida*). Root length was increased by 7.2%, 4.6%, and 1.1% with the applicaitons of ZE-7+12, ZE-7+13, and ZE-12+13, respectively. The highest effect on stem diameter (10.2% increase) was noted for ZE-12+13 (*Pseudomonas putida* + *Acinetobacter calcoaceticus*). The highest impact on number of fruits (13.9% increase) was observed for ZE-12+13. The application of ZE-2 increased plant fresh weight by 8.8%, whereas ZE-7+13 caused a 1.9% increase. The other treatemnts did not significantly affect plant fresh weight. The highest effect on plant dry weight was observed for ZE-7+12, while ZE-13 and ZE-12+13 increased root dry weight by 15.9%, ZE-7+12 by 15.4%, ZE-7+12+13 by 9.5%, ZE-7+13 by 8.1%, ZE-2 by 6.9%, and ZE-5 by 4.7%. The most increase (9.3%) in root fresh weight was recorded for ZE-13 (*Acinetobacter calcoaceticus*) (Figure 1).

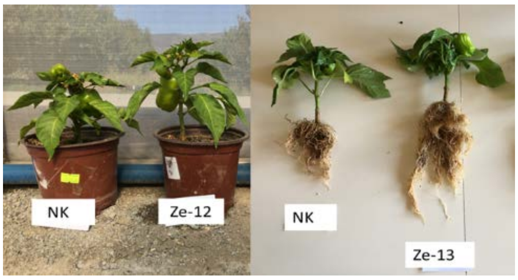

Figure 1. Images from pot experiments.

3.2. Field trials

The highest increase in plant height (12.3%) was observed with the application of ZE-12. Root length was improverd by 11.8% with the application of ZE-7+12 followed by ZE-7 and ZE-12 with 8.1% and 5.9% increase, respectively (Figure 2). The highest increase in stem diameter (16.07%) was observed with the application of ZE-13. Similarly, the highest increase in number of fruits (40.5%) was noted for ZE-12+13. In the same way, ZE-12 application caused 37.2% increase in plant fresh weight. Similarly, ZE-12 application increased plant dry weight by 30.8%.

Root fresh weight was improved by 29.2% with the application of ZE-12. Finally, ZE-7+12 increased root dry weight bu 32.4%, which was significantly different from the control group (Table 4).

Figure 2 Images from the field trials.

Generally, non-significant differences were observed among the treatemnts for evaluated plant traits in pot and field experiments. Nevertheless, root dry weight was statistically significantly different from the control in the field trial. Similar findings were reported by Shrestha et al. (2014), where lactic acid bacteria did not show a significant effect on plant growth in either greenhouse or field studies. While some treatments had negative effects in the pot experiment, these effects were not observed in the field study. This difference may be attributed to several factors, including soil characteristics that support plant growth, nutrient composition of the soil, root colonization, environmental parameters, competition, the production of antagonistic metabolites, and changes in climate conditions (such as temperature and humidity).

Although statistically non-significant differences was observed between the bacterial treatmens and control group for plant growth traits (i.e., plant height, stem diameter, fruit number, plant fresh weight, plant dry weight, root fresh weight, and root dry weight) exhibited some improvement in the field trial. Previous studies have reported that *Pseudomonas putida* increases siderophore production, *Bacillus cereus* degridates phosphate, and *Acinetobacter calcoaceticus* has properties like indole acetic acid production, phosphate reduction, and nitrogen fixation (Santoyo et al., 2012; Kang et al., 2012; Felipe et al., 2021). Furthermore, Pseudomonas and Bacillus species are known to synthesize auxins, multiply rapidly, produce antibiotics, and bacteriotoxins. They also activate systemic resistance and enhance secondary metabolite production, which could explain the positive effects observed in the pepper plants in this study.

A similar study on peanuts reported that *Bacillus* spp. and *Pseudomonas* spp. (specifically *Bacillus velezensis* strains RI3 and SC6, and *Pseudomonas psychrophila* strain P10) applied as PGPR improved plant biomass by 12-18%. This increase was attributed to higher levels of auxins (22%), gibberellins (23%), and cytokinins (27%) (Bigatton et al., 2024). This supports the idea that the positive growth effects in pepper plants could be due to the similar mechanisms exhibited by the bacterial strains used in the current study. Khodabin et al. (2023) investigated the effects of plant growth-promoting bacteria such as *Bacillus subtilis, Azospirillum lipoferum, Azotobacter chroococcum, Enterobacter cloacae*, and *Pseudomonas putida* on soil properties, nutrient uptake, and growth of bell peppers, chemical and biological properties of the soil, NPK uptake, growth, and yield characteristics. It was observed that these bacteria increased the uptake of total nitrogen, phosphorus, and potassium in the soil. The highest nitrogen content in the soil (0.44%), nitrogen uptake (28.8 g/m^2), and shoot dry weight (0.565 g/m^2) were noted for *Azotobacter chroococcum* application. The highest rhizospheric microbial population (7.6×10^6 cfu/g^{-1}) and R/S ratio of 47.9 were recorded with *Enterobacter cloacae*, and this treatment also resulted in higher yield (18.8 kg/m^2) and fruit number (65/8/m^2) (Khodabin et al., 2023). These bacteria were suggested to be used as bio-fertilizers.

In the current study, *E. cloacae* strain (ZE-2) increased number of fruits by 5.5% and plant fresh weight by 8.8% in pot experiment, while *P. putida* increased number of fruit by 5.5%. Khodabin et al. (2023) reported that *P. putida* increased number of fruits by 34% and plant fresh weight by 37.2% in the field tials. These findings suggest that beneficial bacteria can enhance plant growth and yield, which is consistent with the results of this study. The similarity between the findings of current study and previous studies further supports the potential use of these bacteria as growth-promoting agents in pepper cultivation.

Table 3. Effect of bacterial isolates on plant growth in healthy pepper plants (Pot study)

App.	Plant Height		Root Length		Stem Diameter		Fruit Number		Plant Fresh Weight		Plant Dry Weight		Root Fresh Weight		Root Dry Weight	
	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. piece	% Effect	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. piece	% Effect
NC	17.2^{a}	0	22.3^{a}	0	2.6^{a}	0	3.4^{a}	0	21.0^{a}	0	2.6^{a}	0	30.9^{a}	0	2.9^{a}	
ZE-2	17.1^{a}	-0.6	22.1^{a}	-1.1	2.8^{a}	5.4	3.6^{a}	5.5	23.1^{a}	8.8	2.5^{a}	-3.6	30.0^{a}	-2.6	3.1^{a}	6.9
ZE-5	17.6^{a}	2.5	22.2^{a}	-0.3	2.7^{a}	1.5	3.6^{a}	4.2	18.3^{a}	-14.9	2.6^{a}	1.2	30.0^{a}	-3.2	2.9^{a}	4.7
ZE-7	16.5^{a}	-4.4	20.2^{a}	-0.3	2.7^{a}	3.6	2.9^{a}	-17.2	20.2^{a}	-3.9	2.2^{a}	-15.8	25.6^{a}	-21.2	2.7^{a}	-4.4
ZE-8	15.3^{a}	-12.6	21.4^{a}	-4.05	2.8^{a}	5.4	3.4^{a}	-1.5	16.0^{a}	-31.2	2.1^{a}	-23.6	24.8^{a}	-25	2.3^{a}	-21.7
ZE-12	17.8^{a}	3.9	20.8^{a}	-7.05	2.7^{a}	2.2	3.6^{a}	5.5	16.1^{a}	-30.7	2.2^{a}	-15.3	30.4^{a}	-1.8	2.8^{a}	-2.1
ZE-13	17.1^{a}	-0.2	22.5^{a}	0.8	2.8^{a}	6.4	3.6^{a}	4.2	16.4^{a}	-28.7	2.5^{a}	-2.8	34.2^{a}	9.3	3.4^{a}	15.9
ZE-7+12	20.3^{a}	15.2	24.0^{a}	7.2	2.7^{a}	4	3.8^{a}	10.5	20.4^{a}	-3.1	3.1^{a}	16.3	32.3^{a}	4.02	3.4^{a}	15.4
ZE-7+13	19.3^{a}	10.8	23.4^{a}	4.6	2.8^{a}	7.7	3.9^{a}	12.8	21.5^{a}	1.9	2.9^{a}	11.7	30.2^{a}	-2.61	3.1^{a}	8.1
ZE-12+13	18.5^{a}	7.03	22.6^{a}	1.1	2.9^{a}	10.2	3.9^{a}	13.9	19.2^{a}	-9.5	2.9^{a}	10.8	31.8^{a}	2.4	3.4^{a}	15.9
ZE-7+12+13	17.1^{a}	-0.3	21.3^{a}	0	2.8^{a}	7.7	3.7^{a}	6.8	16.9^{a}	-24.3	2.7^{a}	7.2	28.1^{a}	-10.2	3.2^{a}	9.5

*The difference between values with the same letter in the same column is statistically non-significant. (p>0.05). (ZE-2: *Enterobacter cloacae*, ZE-5: *Enterobacter aerogenes*, ZE-7: *Bacillus cereus*, ZE-8: *Microbacterium testaceum*, ZE-12: *Pseudomonas putida*, ZE-13: *Acinetobacter calcoaceticus*).

Table 4. Effect of bacterial isolates on plant growth in healthy pepper plants (Field study)

App.	Plant Height		Root Length		Stem Diameter		Fruit Number		Plant Fresh Weight		Plant Dry Weight		Root Fresh Weight		Root Dry Weight	
	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. piece	% Effect	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. (cm)	% Effect	Av. piece	% Effect
NC	61.2^{a}	0	21.1^{a}	0	4.9^{a}	0	11.5^{a}	0	283.6^{a}	0	64.3^{a}	0	32.3^{a}	0	8.1^{a}	0
ZE-7	69.2^{a}	11.5	22.9^{a}	8.1	5.8^{a}	14.6	11.9^{a}	34	426.2^{a}	33.5	89.9^{a}	28.5	42.9^{a}	24.8	11.2^{ab}	27.5
ZE-12	69.8^{a}	12.3	22.5^{a}	5.9	5.9^{a}	16.3	17.4^{a}	34	451.8^{a}	37.2	92.9^{a}	30.8	45.6^{a}	29.2	11.6^{ab}	29.5
ZE-13	65.7^{a}	6.9	21.5^{a}	1.9	5.9^{a}	16.7	16.7^{a}	31.2	422.8^{a}	32.9	89.0^{a}	27.8	44.5^{a}	27.4	10.9^{ab}	25.7
ZE-7+12	68.8^{a}	11.1	23.9^{a}	11.8	5.8^{a}	14.9	13.9^{a}	17.3	407.5^{a}	30.4	84.7^{a}	24.1	45.2^{a}	28.6	12.0^{b}	32.4
ZE-7+13	68.5^{a}	10.7	22.2^{a}	4.95	5.7^{a}	14.1	17.0^{a}	32.6	419.9^{a}	32.5	88.4^{a}	27.3	39.8^{a}	18.8	10.0^{ab}	18.9
ZE-12+13	64.5^{a}	5.1	22.5^{a}	5.9	5.4^{a}	8.0	19.3^{a}	40.5	375.2^{a}	24.4	76.0^{a}	15.4	33.4^{a}	3.23	8.6^{ab}	5.02
ZE-7+12+13	64.9^{a}	5.8	23.0^{a}	8.3	5.5^{a}	9.5	18.7^{a}	38.6	373.4^{a}	24.1	87.5^{a}	26.5	41.9^{a}	22.9	10.4^{ab}	22.1

*The difference between values with the same letter in the same column is statistically non-significant. (p>0.05). (ZE-2:*Enterobacter cloacae,* ZE-5:*Enterobacter aerogenes,* ZE-7:*Bacillus cereus,* ZE-8:*Microbacterium testaceum,* ZE-12: *Pseudomonas putida,* ZE-13*: Acinetobacter calcoaceticus)*

4. Conclusion

The effects of PGPR (Plant Growth-Promoting Rhizobacteria) applications on growth traits of pepper plants (plant height, root length, stem diameter, number of fruits, plant fresh and dry weight, and root fresh and dry weight) remained non-significant compared to the negative control. However, plant growth traits generally showed positive responses to the PGPR applications in both pot and field trials.

Acknowledgements

This study was supported by Tokat Gaziosmanpaşa University Scientific Research Project Unit (Project No: 2016/77).

Conflict of interest

The authors declare no conflicts of interest.

Authorship contribution statement

Z.K: Writing-Original draft preparation, conducting the experiment, taking observations and measurements reviewing and editing. S.B: Conducting the experiment, taking observations and measurements, reviewing and editing. Y.Y: Planning and conducting the experiment, performing statistical analyses, Reviewing and Editing. M.M: Planning and conducting the experiment, reviewing and editing.

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