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Research Article

FIELD TRIAL OF PGPR, *Bacillus megaterium* E-U2-1, ON SOME VEGETABLE SPECIES

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ABSTRACT. Excessive and unconscious use of chemical fertilizers and pesticides in crop production causes deterioration of soil health, environmental pollution, and an increase in the population of pathogens and pests in the soil. There are many toxic and dangerous chemicals in agricultural ecosystems, and they get into plants, soil, groundwater, and food. As an alternative way to solve this problem, it is very important to use beneficial microorganisms, to use biological agents instead of chemicals in agriculture, and to promote the resistance to biotic and abiotic stress conditions and to increase the yield of plants. Microbial fertilizers, which are becoming increasingly widespread in plant production, are produced from formulations containing *Bacillus megaterium*, especially from Bacillus group bacteria. In this study, *Bacillus megaterium*, E-U2-1 (OL673801 EU. U21-NCBI accession code) strain carrying phytase, phosphatase, siderophore, and indole acetic acid encoding genes was used for the trials. A field trial was carried out with some vegetable species (pepper, eggplant, cucumber, watermelon, melon, and snake cucumber) with high economic value. In the study, 5 ml, 10 ml, and 15 ml of bacterial suspensions with 3x107 cfu were applied per plant from the soil. It was determined that it increased vegetative growth and shortened the flowering period in all species. The effect of the bacterial application on the important agronomic properties of vegetables was investigated, and it was determined that especially 10 ml and 15 ml applications had a positive effect on yield and quality parameters. A significant increase was recorded vegetatively, and while flowering was observed in the earlier period, it was determined that the number of flowers increased considerably. Accordingly, the fruit setting rate increased.

Keywords: *Vegetable, Bacillus megaterium, biofertilizer, biostimulant.*

INTRODUCTION

The increasing need for food due to the rapid increase in the world population has led the agricultural sector to produce higher yields per unit area. Efforts to obtain higher yields per unit area have caused more intensive use of agricultural technologies and chemicals, and accordingly, environmental pollution has increased rapidly and reached dimensions that adversely affect human health. However, there are different approaches to reducing the negative effects of excessive agrochemical use. One of them is the use of beneficial micro-organisms such as mycorrhiza and PGPR (plant growth promoting rhizobium) in plant production. In recent years, researchers have been carried out on the use of beneficial microorganisms, which are important tools for sustainable agriculture,

in areas such as biocontrol of plant diseases, plant growth promotion, and biofertilization. [1]. The most important beneficial microorganisms used in plant production are bacteria [2, 3, 4, 5]. Bacteria play an important role in the soil microflora ecosystem and are among the most complex and diverse communities in the biosphere [6].

Positive effects of plant growth promoting bacteria (PGPB) applications on plant growth (germination rate, leaf area, chlorophyll and protein content, nitrogen content, root and stem development, root and stem weight biomass, yield, and drought resistance) were reported [7, 8, 9]. In addition, some of the beneficial effects of PGPRs can be listed as improving seed germination, supporting root growth, increasing crop yield and natural soil fertility, reducing pollutant toxicity, and protecting against plant pathogens and environmental stress. It can also reduce the global dependence on dangerous agro-inorganic chemicals that destabilize agro-ecosystems. Therefore, they will play a pivotal role in sustainable crop production and the environment as bio-fertilizer in the future [10].

Bacteria with PGPR properties have both direct and indirect effects on plant growth and health. These bacteria can act directly by fixing nitrogen, supporting the production of plant hormones such as auxin, cytokinin, gibberellin, facilitating the uptake of iron and similar trace elements with the production of bacterial siderophores, dissolving mineral and organic phosphates, and converting other nutrients into forms that the plant can uptake easily. Or, as mentioned above, they are known to indirectly promote plant growth by suppressing plant disease factors [7, 11, 12].

These root bacteria, which stimulate plant growth, are generally found in genera such as *Bacillus, Lactobacillus, Paenibacillus, Arthobacter, Pseudomonas, Burkholderia, Enterobacter, Pantoae, Klebsiella, Xanthomonas, Serratia, Rhizobium, Bradyrhizobium, Azospirillium, Azotobacter* [8].

Among them, *Bacillus* is rod-shaped, Gram-positive, obligate or facultative aerobic and motile bacteria. *Bacillus* bacteria can produce different extracellular enzymes such as amylase, lipase, chitinase, xylanase, pectinase, protease, and cellulase. *Bacillus* species can have a direct effect on plant growth through the synthesis of plant growth hormones [13, 14], nitrogen fixation [15], and the synthesis of enzymes that regulate the level of rhizobacteria that promote plant growth [16, 17].

Bacillus megaterium, one of the important species among *Bacillus* bacteria, is a rod-like, Gram-positive, mostly aerobic spore-forming bacterium found in a wide variety of habitats. Cell length up to 4 μm and diameter 1.5 μm. *B. megaterium* is among the largest known bacteria. Cells are often found in chains where cells are interconnected by polysaccharides in their cell walls and synthesize a capsule consisting of both a polypeptide and a polysaccharide. It is considered aerobic but can also grow under anaerobic circumstances as needed. A saprophyte is an organism, it recycles organic matter in the soil and converts it into simpler substances that can be absorbed by other organisms. *B. megaterium* can survive even in harsh conditions such as desert environments due to the spores it produces.

The benefits it provides to plants can be summarized as follows; 1. *B. megaterium* can produce numerous organic acids during their growth and reproduction and organic acid can decompose or dissolve insoluble phosphorus substances in soil and convert them into phosphorus, which is easily absorbed by plants; 2. *B. megaterium* can become an advantageous bacterium in the rhizosphere because it can multiply very quickly in the soil and control the nutrition in roots and other sources. Thus, blocking the habitat and condition of pathogenic bacteria, and creating a barrier against virus attack; 3. *B. megaterium* can increase plant root volume and provide effective plant nutrient and water

uptake. In studies on beneficial microorganisms, sustainability needs to identify local beneficial bacteria strains and bring them to the agricultural sector. Recently, studies are carried out to determine *B. magetrium* strains with plant growth-promoting properties and to determine their interactions with different plant species. In this study, it was aimed to reveal the effect of local *B. megaterium* strain E-U2-1 carrying the genes encoding phytase, phosphatase, siderophore, and indole acetic acid. Isolates carrying siderophore and acid phosphatase (AcPho) genes are effective in promoting plant growth. Some isolates of *Bacillus thuringiensis* can provide indole acetic acid. Due to these properties, it has been stated that it can be an important bioinoculant that provides soluble iron and phosphorus to the plant [41, 42]. In this study, the effects of *B. megaterium* (E-U2-1(OL673801 EU.U21-NCBI accession code)) on agronomic characteristics of commercially important vegetable species such as watermelon, melon, cucumber, snake cucumber, pepper and eggplant were investigated under open field conditions.

MATERIALS AND METHODS

Collection of Soil Samples

A cross-sectional study was conducted in the province of Kayseri, located in the interior part of Turkey. Sixty different soil samples were collected from 16 districts in different locations of Kayseri province at altitudes of 1050-1680 meters. Soil samples were sampled from the rhizosphere soil of the alfalfa plant (*Medicago sativa* L.) between May and August 2021. Soil samples taken from 0-30 cm depth of the rhizosphere were stored in sterile 50 ml falcon tubes at 4 °C for further analysis.

Bacteria Isolation from Soil

Net five grams of soil from the soil samples brought to the laboratory under aseptic conditions were placed in sterile containers. The samples were then transferred in two stages into flasks containing 50 mL sterile water (0.9% NaCl) and Luria- Bertani (LB) Broth medium. To full homogenization, samples in the LB media were kept in a shaking incubator for 4-6 hours while samples in the sterile water were kept for 30-40 min. After incubation, 10 solutions were taken from each sample and plated on solid LB and NB mediums by the spread plate method. The plated samples were then incubated at 35 ºC for 12-24 hours and bacteria main cultures were obtained. At the end of the incubation period, isolates of different colonies from the main culture in the petri dish were selected and purified. Purified strains were incubated and transferred to NB media. Each isolate was then given a code name [18, 19]. Stock culture of the purified isolates were taken in a solution containing 800 µl of LB and 200 µl of 50% glycerol and were preserved in -20 ºC for further analyses.

DNA Isolation from Bacteria

Bacterial isolates were inoculated in 10 mL of Nutrient broth medium and grown for 24 hours at a shaking speed of 150 rpm at 28±2 °C. Then, 1.5 mL of bacterial culture was transferred into sterile microcentrifuge tubes and centrifuged at 10000xg for 5 minutes to precipitate bacterial cells. The supernatants were discarded, and cell precipitates were dissolved in 567 µl of Tris EDTA buffer with a pH of 8.0. 30 µl of 10% SDS and 3 µl of proteinase K were added to the precipitates and mixed thoroughly. Samples were then incubated at 37 °C for 1 hour. After incubation, 100 µl of 5M NaCl was added to each

sample and then treated with 80 µl of CTAB/NaCl solution. The samples were mixed thoroughly by pipetting until white precipitates formed and incubated at 65 °C for 10 minutes. At the end of the incubation period, 0.75 ml of chloroform/isoamyl alcohol (24/1 volume/volume) was transferred into each microcentrifuge containing the samples and mixed thoroughly. The supernatants were transferred into new microcentrifuge tubes and centrifuged at 10000xg for 10 minutes. 0.75 ml of phenol/chloroform/isoamyl alcohol (25/24/1 vol/vol) was added to the upper phase and centrifuged at 10000xg for 10 minutes. At the end of the centrifugation process, the supernatants in the tubes were transferred to new microcentrifuge tubes, and 0.6 µl of the total volume (~350 µl) of isopropanol was added to the samples and mixed thoroughly by inverting the tubes back and forth until a white precipitate formed. After centrifuging at 10,000xg for 5 minutes, the supernatants were discarded and 350 µl of 70% ethanol was added to the precipitate and centrifuged again at 10,000xg for 5 minutes. The supernatants were carefully discarded, and the chromosomal DNA samples were left to dry at room temperature. In the final step, the DNA was treated in 100 µl of TE buffer for approximately 1 hour to dissolve [20, 21].

PCR Amplification and Sequencings of 16S rDNA Gene

The 16S rDNA regions in the genomic DNAs were determined and amplified in a thermal cycler (Techne- TC-512, England) using universal 16S forward (5'- AGA GTT TGA TCC CTC AG -3') and 16S reverse (5'- CCG TCA ATT CCT TTG AGT TT -3') primers. In an orderly manner, 16.9 µl of sterile water, 2.5 µl of 10xPCR buffer, 0.5 µl (10 nM) of dNTP mix, forth and reverse primers (0.3 pM), 2 µl (2.3 mM) of $MgCl_2$, 0.20 µl (0.5U) of Taq DNA, 2.5 µl (30 ng) of genomic DNA, making a total volume of 25 µl were mixed thoroughly by pipetting. Subjected to PCR reaction follows: DNA initial denaturation step of 3 min at 95 °C, 25 cycles for denaturation at 94 °C for 1 min, melting temperature1 min cycle (at 50 °C for AC Phosphatase, 52 °C for Siderophore, 53 °C Phytase and 57 °C Indole Acetic Acid) and 1 min at 72°C. Finally, the PCR protocol was maintained at 72 °C for 10 min for the elongation step. The resulting PCR products were run on a gel containing 1% agarose, and DNA fragments containing the 16S rDNA gene were extracted from the gel.

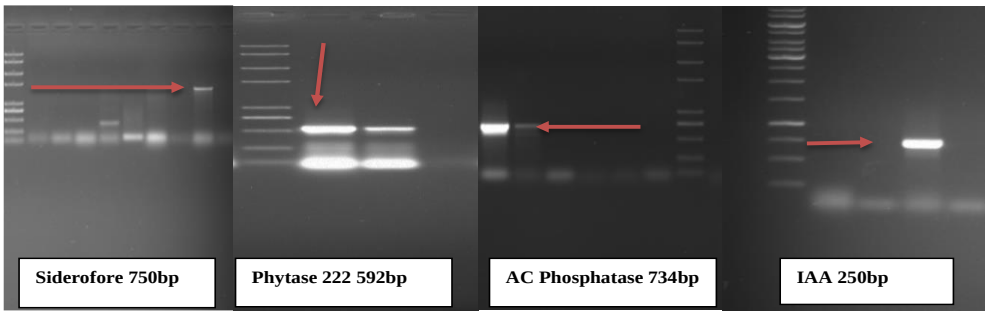

Fig 1. *PCR gel images of Bacillus megaterium siderophore, phytase, phosphatase, and indole acetic acid gene regions*

Bacteria Identification

After 16s PCR DNA analysis, gel bands were cut under UV light and stored at -20 ℃. According to the manufacturer's instructions, DNA fragments for sequencing were extracted from the gel using the Rapid Gel Extraction Kit (MG-JEK-01-50). Then, PCR products of 24 samples with the clearest bands were sent for Sanger dideoxy sequencing. Sequencing was done through service procurement. Also, MALDI-TOF analysis through service procurement was performed for confirming identification of isolates in species level [22, 23, 24, 25, 26, 27, 28].

Field Studies of The Bacterium

Bacillus megaterium strain E-U2-1 (OL673801 EU.U21-NCBI) accession code determined as PGPR containing four related genes was applied to six different vegetables species. Bacterial isolate was applied to melon, watermelon, cucumber, snake cucumber, eggplant and pepper under open field conditions. *B. megaterium* strain E-U2-1 bacteria ($3x10^7$ cfu) were applied per plant in soil and three different doses (5 ml, 10 ml, 15 ml). The strain was applied to the soil 3 weeks after transplanting. The location of the experimental field is Üçkuyu village, Avanos County, Nevşehir province at an altitude of 920 m. The experimental design was randomized plots experimental design with 3 replications, 3 doses and control groups for each species. Cultural practices were carried out regularly and water was given three times a week with drip irrigation. Plant height/length (PH/PL), the number of flowers (FLN), the number of branches (BN) and the number of fruits (FN) were determined.

Data Analysis

The data were analyzed with the SAS statistical program version 9.00 (SAS Institute, USA) using the GLM procedure (PROC GLM) and mean separation was conducted with DUNCAN at α=0.05/0.01 for significant level ($p \leq 0.05/0.01$). ANOVA was performed to determine the effects of bacterial isolate on vegetable species on certain agronomic traits. The results obtained in the agronomic properties studies were evaluated by performing variance analysis in the SAS statistical program.

RESULTS AND DISCUSSION

Solanaceae Family

The effects of bacterial applications on agronomic properties were determined in pepper and eggplant species belonging to the *Solanaceae* family. It has been determined that *B. megaterium* E-U2-1 bacterial applications affect yield and quality positively in pepper. It was determined that after bacterial applications, plant height increased rapidly compared to the control group. Especially in the Dose-2 (10 ml) application, it was observed that the plant height of pepper increased regularly and rapidly. When the flowering status of pepper was examined, it was determined that bacterial applications were effective in blooming at an earlier period. From the first week after dose-1 (5 ml) application, flowering started intensively.

The total number of flowers was significantly affected by the bacteria application, and the number of flowers was determined more than the control in all three doses of bacteria application. The increase in the number of flowers per plant ranged from 31 flowers/plant

to 16 flowers/plant 5 ml bacteria application had the highest number of flowers with 54 flowers, while control plants had 23 flowers. It is seen that bacteria applications have a significant effect on both earliness and yield in pepper. The dose-1 application was found to be statistically significant compared to other applications and the control group. In pepper, the highest plant development and branching were recorded in dose-2 application, while the highest fruit setting rate and continuity were found in dose-3 application (Table 1). The difference in the number of fruits per plant was not found to be significant. Similar to the current study in pepper, it was reported that root and stem fresh weight, root diameter, root fresh weight and dry weight were increased by bacterial applications [29, 30].

In another study examining the effects of different bacterial formulations on plant growth, yield and mineral substance content in pepper, 3 different formulations of microbial fertilizers prepared from Bacillus isolates (*Bacillus megaterium* (TV-87A), *Bacillus megaterium* (TV-91C), *Bacillus megaterium* (M3), *Paenibacillus polymyxa* (TV-12E), *Pantoea agglomerans* (RK-92), *Bacillus subtilis* (TV-17C), *Bacillus pumilus* (TV-73A)) were used as PGPR. It was stated that the yield parameters of the plant increased [31].

In eggplant, on the other hand, it was determined that the plant height increased rapidly with bacterial applications. Plant development increased in eggplant seedlings each week during the first month after application. A statistically significant difference was found in bacterial applications compared to the control group. The best and continuous vegetative growth was obtained from the Dose-3 application. Similarly, flowering increased from the first week after bacterial application, and it was found to be statistically significant compared to the control group. A total of 30 flowers were recorded in the dose-1 application, 20.6 flowers in the dose-2 application, 28.3 flowers in the dose-3 application, and 12 flowers in the control group, within a month following the bacterial application. Considering the branching of the plant, the dose-3 application significantly increased the number of branches compared to other applications and the control group. (Table 2). As in pepper, the difference in the number of fruits per plant in the first month of the experiment was not significant. In different previous studies, it has been determined that microbial fertilizers have positive effects on plant growth and development. Kandil [32] reported that the effects of microbial fertilizer applications on the germination percentage, average germination time and germination index of eggplant (*Solanum melongena* L.) seeds were more successful than the control group, and the germination time was shortened positively. In eggplant seedling, hypocotyl and epicotyl length, the number of leaves, plant fresh weight and dry weight increased with bacterial applications. In another study, it was determined that *B. megaterium* M-3 bacteria applications significantly increased the content of microelements in tomato fruit compared to the control and additionally increased agronomic properties such as fruit length, fruit width and fruit weight [33]. Similarly, Turan et al. [34] determined that phosphorus dissolving *B. megaterium* bacteria application increased the yield and uptake of phosphorus, iron, zinc and copper.

Cucurbitacea Family

After the application of *B. megaterium* E-U2-1 bacteria in cucumber, a rapid increase in the number of flowers was recorded in the first week. In the dose-3 application, the number of flowers and subsequent fruit setting was found to be statistically significant compared to the control group. It was determined that the bacterial applications had a

significant effect on the growth rate and the number of branches of the plant. In dose-2 and dose-3 applications, the number of branches showed a stronger development from the first week (Table 3). In a similar study, it was emphasized that *B. megaterium*-GC subgroup-A MFD-2 treatments on cucumber had positive effects on plant height, the number of fruits per plant, fruit weight per plant, fruit width and fruit length, and soluble solid of fruit [35]. Seymen et al. [36] examined the effects of different bacterial applications on yield and nutrient uptake in cucumbers and reported that FE-43 and N-17/3 bacteria applications positively affected total yield.

Watermelon was significantly affected by bacteria application, and this effect was more pronounced in the second week. A statistically significant increase in plant main stem length was observed in dose-1 and dose-3 applications. In dose-3 application, flowering increased after the second week and fruit formation occurred earlier. *B. megaterium* E-U2-1 bacteria application accelerated vegetative growth and branching and caused early flowering in watermelon. (Table 4). Similarly, plant height and the number of branches increased from the second week after the application of *Bacillus megaterium* E-U2-1 bacteria in melon and snake cucumber, and the highest plant height and number of branches were determined in dose-1 and dose-3 applications (Table 5 and Table 6). According to the results obtained in this study, bacterial applications accelerate vegetative growth and provide significant advantages in earliness in species belonging to the *Cucurbitaceae* family. Similar results were obtained in different studies. In studies carried out on watermelon, investigating the effects of different bacterial combinations, it was reported that there was a significant increase in yield parameters and nutrient intakes with bacterial applications compared to control groups [37, 38]. It has been stated that the applications of growth regulator bacteria in different melon cultivars increased yield and quality. It has been determined that it increased the average fruit yield per plant by 39% and the average fruit weight by 22% [39].

It was reported that *B. megaterium* is one of the known phosphorus solvent bacteria [40]. *Bacillus megaterium* is a bacterium that converts phosphorus components in the soil, which cannot be taken up by the plant, to the form that the plant can take, and also helps to protect plants from soil-borne diseases. *Bacillus megaterium* dissolves bound phosphate forms in the soil, making phosphorus available to plants. In this way, root growth is stimulated and root development is encouraged. It creates more and longer roots in the plant. It increases the ability of plants to absorb water and nutrients. With the taken phosphorus, stronger flower, pollen and fruit formation is ensured. In this study, higher number of flower and early flower and fruit formation were observed as previously reported.

Table 1. *Effects of Bacillus megaterium E-U2-1 application on some agronomical parameters of pepper*

	Pepper																		
	Before Application			**First Week**				**Second Week**				**Third Week**				**Fourth Week**			
	PH	**FLN**	**BN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**
DOSE 1	19.00	2^{ab}	1.00^{c}	27.33^{a}	12.3^{a}	2.67^{b}	4.33^{c}	29.0^{ab}	12.67^{a}	3.00^{b}	5.66^{b}	29.0^{ab}	10.0^{ab}	3.0^{b}	7.0^{b}	29.0^{ab}	4.33^{ab}	3.0^{b}	7.0^{a}
DOSE 2	20.67^{a}	4^{a}	2.00^{ab}	31.27^{a}	8.33^{b}	5.33^{a}	12.3^{b}	34.66^{a}	9.67a	6.00^{a}	9.67^{ab}	34.67^{a}	12.33^{a}	6.0^{a}	16.0^{a}	34.67^{a}	8.33^{a}	6.0^{a}	12.66^{a}
DOSE 3	19.67^{a}	2.6^{ab}	2.67^{a}	27.67^{a}	8.33^{b}	4.33^{a}	18.3^{a}	29.83^{ab}	14.67a	4.66ab	14.33^{a}	29.83^{ab}	9.0^{ab}	4.67^{ab}	19.0^{a}	29.83^{ab}	5.33^{ab}	4.67^{ab}	11.66^{a}
Control	19.67^{a}	1^{ab}	1.67^{bc}	25.33^{a}	5.0^{b}	3.0^{b}	13.3^{ab}	26.33^{b}	13.33^{a}	$3.^{0\ b}$	13.67^{a}	26.33^{b}	2.33^{b}	3.0^{b}	16.67^{a}	26.33^{b}	1.33^{b}	3.0^{b}	11.66^{a}
LSD	5.32	1.96	0.77	7.13	4.33^{b}	1.33	5.43	7.52	7.73	2.17	6.87	7.52	9.26	2.17	7.37	7.51	5.75	2.17	8.83
CV	14.32	43.06	22.27	13.58	23.09	18.45	23.89	13.33	33.95	27.71	33.71	13.26	58.41	27.71	26.69	13.32	63.21	27.71	43.63

*Plant height (PH), number of flowers (FLN), number of branches (BN), and number of fruits (FN)

Table 2. *Effects of Bacillus megaterium E-U2-1 application on some agronomical parameters of eggplant*

	Eggplant																		
	Before Application			**First Week**				**Second Week**				**Third Week**				**Fourth Week**			
	PH	**FLN**	**BN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**
DOSE 1	20.33^{a}	1.66^{a}	1.33^{a}	39.0^{a}	5.0^{a}	2.0^{a}	1.0^{b}	43.33^{a}	7.33^{a}	3.0^{ab}	1.0^{a}	43.33^{a}	8.66^{ab}	3.0^{ab}	2.66^{a}	43.33^{a}	7.33^{ab}	3.0^{ab}	2.0^{a}
DOSE 2	21.33^{a}	0.66^{a}	1.66^{a}	37.66^{a}	2.66^{ab}	2.0^{a}	2.0^{ab}	42.0^{a}	4.33^{a}	3.0^{ab}	1.66^{a}	42.0^{a}	9.0^{a}	3.0^{ab}	1.0^{a}	42.0^{a}	3.66^{b}	3.0^{ab}	1.66^{a}
DOSE 3	19.0^{a}	1.00^{a}	2.66^{a}	42.0^{a}	5.33^{a}	3.66^{a}	3.33^{a}	46.0^{a}	5.67^{a}	5.33^{a}	2.33^{a}	46.0^{a}	7.66^{ab}	5.33^{a}	3.0^{a}	46.0^{a}	8.66^{a}	5.33^{a}	2.33^{a}
CONTROL	19.0^{a}	1.00^{a}	1.0^{a}	37.33^{a}	1.0^{b}	2.0^{a}	1.67^{b}	37.5^{a}	3.0^{a}	2.66^{b}	2.0^{a}	37.5^{a}	3.0^{b}	2.66^{b}	0.33^{a}	37.5^{a}	4.0^{ab}	2.66^{b}	1.33^{a}
LSD	2.43	1.53	1.63	6.22	2.93	1.72	1.54	9.35	5.57	2.24	1.54	9.36	5.77	2.24	2.92	9.35	4.98	2.24	1.33
CV	6.48	75.37	51.96	8.42	44.42	37.77	40.82	8.35	58.19	34.0	46.66	8.35	43.32	34.0	88.83	8.35	44.72	34.0	38.57

*Plant height (PH), number of flowers (FLN), number of branches (BN), and number of fruits (FN)

Table 3. *Effects of Bacillus megaterium E-U2-1 application on some agronomical parameters of cucumber*

	Cucumber																	
	BEFORE APPLICATION			First Week				Second Week				Third Week				Fourth Week			
	PH	FLN	BN	PH	FLN	BN	FN	PH	FLN	BN	FN	PH	FLN	BN	FN	PH	FLN	BN	FN
DOSE 1	36.0^{b}	4.0^{c}	1.0^{a}	52.66^{b}	13.66^{b}	1.33^{a}	2.0^{c}	85.0^{a}	30.0^{a}	1.66^{c}	2.66^{b}	85.0^{a}	43.0^{a}	1.66^{c}	3.0^{b}	86.0^{a}	34.67^{a}	1.66^{c}	2.0^{a}
DOSE 2	74.0^{a}	17.0^{a}	2.0^{a}	74.0^{b}	27.0^{ab}	3.66^{a}	3.33^{b}	76.0^{a}	33.67^{a}	4.66^{a}	3.66^{b}	76.0^{a}	52.67^{a}	4.66^{a}	3.33^{ab}	77.0^{a}	45.67^{a}	4.8^{a}	1.66^{a}
DOSE 3	68.0^{a}	10.5^{b}	2.0^{a}	85.0^{a}	42.0^{a}	3.66^{a}	5.66^{a}	90.0^{a}	50.67^{a}	4.0^{ab}	6.33^{a}	90.0^{a}	56.0^{a}	4.0^{ab}	4.66^{a}	90.0^{a}	47.33^{a}	4.2^{ab}	3.66^{a}
CONTROL	72.0^{a}	9.0^{cb}	1.0^{a}	82.0^{a}	27.66^{ab}	2.33^{a}	3.66^{b}	86.0^{a}	27.33^{a}	2.33^{bc}	2.66^{b}	86.0^{a}	54.0^{a}	2.33^{bc}	3.33^{ab}	86.0^{a}	25.67^{a}	2.33^{bc}	2.66^{a}
LSD	11.78	5.89	-	15.29	14.87	2.36	0.94	27.27	30.56	2.10	2.17	27.27	32.22	2.10	1.63	27.27	33.97	2.10	3.12
CV	3.79	12.19	-	10.77	28.64	45.75	13.63	15.67	45.83	35.30	30.12	15.67	33.28	35.30	24.17	15.67	47.0	35.30	73.70

*Plant height (PH), number of flowers (FLN), number of branches (BN), and number of fruits (FN)

Table 4. *Effects of Bacillus megaterium E-U2-1 application on some agronomical parameters of watermelon*

	Watermelon																		
	BEFORE APPLICATION			First Week				Second Week				Third Week				Fourth Week			
	PH	FLN	BN	PH	FLN	BN	FN	PH	FLN	BN	FN	PH	FLN	BN	FN	PH	FLN	BN	FN
DOSE 1	91.66^{a}	1.66^{a}	2.33^{a}	134.6^{a}	1.6.0^{a}	4.66^{a}	2.33^{a}	197.33^{a}	4.0^{a}	5.0^{a}	1.66^{a}	197.33^{a}	3.33^{cb}	5.0^{a}	1.0^{a}	197.33^{a}	3.66^{ab}	5.0^{a}	1.33^{a}
DOSE 2	98.0^{a}	3.0^{a}	2.33^{a}	135.6^{a}	3.0^{a}	5.0^{a}	1.66^{a}	161.67^{a}	3.33^{a}	4.66^{a}	1.33^{a}	161.67^{a}	5.33^{b}	4.66^{a}	2.33^{a}	161.67^{a}	1.33^{bc}	4.66^{a}	1.0^{a}
DOSE 3	91.0^{a}	1.66^{a}	2.0^{ab}	136.0^{a}	1.66^{a}	4.66^{a}	1.66^{a}	189.67^{a}	3.67^{a}	5.0^{a}	1.0^{a}	189.67^{a}	10.0^{a}	5.0^{a}	1.33^{a}	189.67^{a}	4.33^{a}	5.0^{a}	2.0^{a}
CONTROL	118.5^{a}	1.0^{a}	1.33^{b}	146.0^{a}	1.0^{a}	4.0^{a}	1.0^{a}	160.0^{a}	4.0^{a}	4.0^{a}	1.0^{a}	160.0^{a}	2.0^{c}	4.0^{a}	1.0^{a}	160.0^{a}	1.0^{c}	4.0^{a}	1.0^{a}
LSD	16.62	3.21	0.94	26.96	3.35	1.54	2.17	49.79	3.72	1.44	2.10^{a}	49.79	2.92	1.43	1.53	49.79	2.87	1.43	2.17
CV	8.27	-	25.0	9.58	-	17.81	76.98	14.92	52.77	16.36	95.83	14.92	30.08	16.36	57.63	14.92	63.21	16.36	86.60

*Plant height (PH), number of flowers (FLN), number of branches (BN) and number of fruits (FN)

Table 5. *Effects of Bacillus megaterium E-U2-1 application on some agronomical parameters of melon*

	Melon																	
	Before Application			**First Week**				**Second Week**				**Third Week**				**Fourth Week**			
	PH	**FLN**	**BN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**
DOSE 1	26.0^{ab}	10.0^{a}	1.33^{b}	57.67^{a}	23.33^{b}	2.00^{a}	1.0^{b}	67.33^{ab}	10.^{b}	4.33^{a}	1.0^{a}	67.33^{ab}	10.33^{a}	4.33^{a}	1.33^{b}	67.33^{ab}	5.33^{a}	4.33^{a}	2.0^{a}
DOSE 2	27.0^{ab}	9.33^{a}	1.66^{b}	38.50^{a}	30.0^{a}	1.66^{a}	3.0^{a}	44.0^{b}	14.0^{a}	2.66^{b}	1.0^{a}	44.0^{b}	8.66^{ab}	2.66^{b}	1.0^{b}	44.0^{b}	4.66^{a}	2.66^{b}	1.66^{a}
DOSE 3	21.33^{b}	7.66^{a}	1.33^{b}	48.0^{a}	21.66^{b}	1.67^{a}	1.0^{b}	75.0^{a}	12.66^{ab}	3.0^{ab}	1.0^{a}	75.0^{a}	9.33^{ab}	3.0^{ab}	2.0^{a}	75.0^{a}	4.66^{a}	3.0^{ab}	2.0^{a}
CONTROL	31.0^{a}	8.33^{a}	3.33^{a}	50.0^{a}	19.33^{b}	2.0^{a}	1.0^{b}	49.67^{ab}	9.33^{b}	3.0^{ab}	1.0^{a}	49.67^{ab}	5.66^{b}	3.0^{ab}	1.0^{b}	49.67^{ab}	4.33^{a}	3.0^{ab}	0.66^{b}
LSD	7.10	3.64	1.08	29.58	5.56	2.43	2.0	27.18	3.21	1.53	-	27.18	3.68	1.53	0.54	27.18	4.55	1.54	0.76
CV	12.09	21.92	30.12	29.21	12.54	70.41	-	24.46	14.85	25.12	-	24.46	23.03	25.12	21.65	24.46	50.84	25.12	25.78

*Plant height (PH), number of flowers (FLN), number of branches (BN), and number of fruits (FN)

Table 6. *Effects of Bacillus megaterium E-U2-1 application on some agronomical parameters of snake cucumber*

	Snake Cucumber																		
	Before Application			**First Week**				**Second Week**				**Third Week**				**Fourth Week**			
	PH	**FLN**	**BN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**	**PH**	**FLN**	**BN**	**FN**
DOSE 1	28.0^{ab}	8.0^{a}	1.0^{a}	33.66^{ab}	23.33^{b}	2.0^{a}	1.0^{a}	39.33^{a}	36.66^{a}	4.0^{a}	2.66^{a}	39.33^{ab}	9.66^{a}	4.0^{a}	2.66^{a}	39.33^{ab}	7.33^{a}	4.0^{a}	2.33^{a}
DOSE 2	29.0^{a}	8.33^{a}	1.0^{a}	37.33^{a}	30.0^{a}	1.67^{a}	0.35^{a}	38.50^{ab}	36.33^{a}	4.33^{a}	2.67^{a}	38.5^{ab}	10.0^{a}	4.33^{a}	2.66^{a}	38.50^{ab}	6.0^{a}	4.33^{a}	2.0^{ab}
DOSE 3	28.66^{a}	7.33^{ab}	1.0^{a}	35.0^{ab}	21.66^{b}	1.67^{a}	0.33^{a}	40.33^{a}	37.66^{a}	3.33^{ab}	2.60^{a}	40.33^{a}	10.66^{a}	3.33^{ab}	3.0^{a}	40.33^{a}	7.33^{a}	3.33^{ab}	2.0^{ab}
CONTROL	24.66^{b}	5.33^{b}	1.0^{a}	29.66^{b}	19.33^{b}	2.0^{a}	0.33^{a}	34.5^{b}	19.33^{a}	2.33^{b}	1.0^{b}	34.5^{b}	5.33^{b}	2.33^{b}	1.0^{b}	34.50^{b}	2.0^{b}	2.33^{b}	1.33^{b}
LSD	3.57	2.10	-	5.56	5.56	2.43	0.77	4.68	20.5	1.33	0.94	4.67	3.64	1.33	0.76	4.67	2.92	1.33	0.76
CV	6.35	15.42	-	8.72	12.54	70.41	97.97	5.44	33.50	20.20	22.22	5.44	21.71	20.20	17.49	5.44	27.43	20.20	21.29

*Plant height (PH), number of flowers (FLN), number of branches (BN), and number of fruits (FN)

CONCLUSION

In recent years, researchers have sought an integrated approach to reduce the negative effects of synthetic chemicals used to increase yield and quality in agricultural production. PGPR works in favor of the plant with many benefits such as protection of soil and water resources, elimination of the negative effects of pesticides and chemical fertilizers that cause environmental pollution, effects on disease and pest control, increasing the uptake of nutrients by the plant, reducing the stress created by biotic/abiotic factors in the plant. The studies on the effects of PGPRs have become widespread and the results are promising. In addition, by increasing the use of bio-products, the input of synthetic chemicals used in agricultural production will be reduced and the production cost will also decrease**.** Studies so far show that PGPRs will largely replace chemicals in agricultural production, with many benefits. Recently, it has been determined that bacterial groups belonging to *Bacillus* species, and *B. megaterium*, which is among them, are important plant growth stimulators.

It was determined that *B. megaterium* E-U2-1, which was previously defined and applied to different vegetables in open-field trials, can be used as a potential biological agent to promote plant growth in the vegetable species used in this study.The use of chemicals can be significantly reduced by the utilization of such bacteria in agriculture to increase yield and quality by developing and using bio-preparations suitable for sustainable agriculture that protect the environment by preventing pollution.

Conflict of Interest. The author declared that there is no conflict of interest.

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