

THE EFFECT OF PLANT GROWTH-PROMOTING BACTERIA ON THE DEVELOPMENT, YIELD AND YIELD COMPONENTS OF BREAD (*TRITICUM AESTIVUM* L.) AND DURUM (*TRITICUM DURUM*) WHEATS

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Abstract. This study was carried out with 3 replications and 24 parcels according to randomized complete blocks trial design between 2017 and 2018 as two separate experiments. The study was planned to determine the effects of previously identified *Stenotrophomonas maltophilia* TV14B (phosphate solubilizing), *Bacillus atropheus* TV83D (nitrogen fixation), *Bacillus*-GC group TV119E (phosphate solubilizing), *Cellulomonas turbata* TV54A (nitrogen fixation) and combined effects of *Bacillus atropheus* TV83D and *Bacillus*-GC group TV119E on the development of Ceyhan-99 and Fırat-93 bread wheat cultivars. According to the results, the highest grain yield for Fırat-93 durum wheat cultivar was obtained under full dose chemical fertilizer application (288.89 kg/da); while the highest grain yield (283.33 kg/da) in the case of bacteria applications was observed with *Bacillus atropheus* TV83D, nitrogen-fixation bacteria. The difference between these two was not significant, and these bacteria can be seen as an alternative to chemical fertilization. The highest yield among bacterial applications was obtained under treatment with *Bacillus atropheus* TV83D, a nitrogen-fixation bacterium with 319.45 kg/da, and the difference between them was statistically significant. At the end of the study it was concluded that *Bacillus atropheus* TV83D bacterial strain can be used as a microbial fertilizer because of its ability to increase grain yield significantly in both genotypes.

Keywords: biofertilizer, diazotrophs, PGP activities, P-solubilization, sustainable production, wheat cultivars

Introduction

Cereals is a large group consisting of economically important species like the *Graminea* (wheats = corbels) family, cool climate cereals like wheat (*Triticum*), barley (*Hordeum*), oats (*Avena*), rye (*Secale*) and triticale, and warm climate cereals like corn (*Zea*), paddy (*Oryza*), maize (*Sorghum*), flint corn (*Panicum*) and avian (*Phalaris*) (Elçi et al., 1994). Wheat, corn and rice generate 85% of the world's grain production. Wheat is the most cultivated grain among these agricultural products providing high energy with grains containing plenty of starch. Wheat (*Triticum* sp.) is the most highly composite among cereals. Wheat is an indispensable plant used as a source of carbohydrates, providing basic input to livestock and industry. It is also the main food source of most countries with the largest cultivation area in the world.

Wheat constitutes approximately 29-30% of the world total grain production and is the most important plant-based protein source of humankind (Gustafson et al., 2009). Scientists consider its genetic complexity as a miracle, and which blends perfectly with changing environmental conditions. Wheat cultivars are the more superior in comparison with other grains and their nutritive values (Zohary and Hopf, 2000).

Global efforts to increase wheat production in parallel with population growth have been partially successful. Although this has also increased the yield of wheat, it is not sufficient to meet the needs of the growing population.

As the aim in crop production is to increase the dry matter yield per unit area, intensive input use increases in parallel. Fertilizer is one of the most important items among these inputs. It is determined that approximately 15% of the total chemical fertilizer used in the world is in wheat farming. There is an increasing trend towards the use of organic fertilizers worldwide to avoid the negative effects of chemical fertilizer use. In these organic fertilizers, Plant Growth-Promoting Bacteria (PGPB) is an alternative source of microbial fertilizers. In researches, especially *Pseudomonas* ssp., *Azospirillum* sp. and *Pantoea* sp. bacteria groups were found to increase plant growth and nitrogen intake in wheat and maize grown in temperate climates (Meena and Rai, 2017).

In recent years, the commercial formulations of these bacteria have been used in the cultivation of many plants such as corn, wheat, potatoes and tomatoes and it has been determined that these bacteria have a significant effect on plant height, root development and increase in dry matter. In addition, the use of these bacteria is predicted to reduce the use, cost and environmental pollution of chemical fertilizers and pesticides (Mehnaz et al., 2001, 2010; Tozlu et al., 2014). It has been reported that bacterial activity varies according to soil properties, soil organic matter content, plant and bacteria variety and plant growing conditions (Çakmakçı et al., 2006). In this respect, further studies are needed on the effects of bacteria on plant growth. In this study, the effect of Plant Growth Inhibiting Bacteria on the development, yield and yield components of wheat were investigated. It has been determined that the effect of the bacteria, which are revealed by field and greenhouse studies especially on wheat, has not been investigated at an adequate level before. In this study, the bacteria, which are isolated from Van Lake Basin and known to promote plant growth, are inoculated in bread and durum wheat varieties. Afterwards, their effects on wheat yield, yield and yield components were investigated.

One of the aims of this study was to quantify the productivity of dryland farmed bread and durum wheat cultivars that was seed inoculated. In addition, the effect of these bacteria on reducing the use of inorganic fertilizers was also examined.

Literature research

In many countries, the effect of Plant Growth-Promoting Bacteria (PGPB) on durability against biotic and abiotic stress on yield increase in cereals has been extensively researched and will continue to be researched more. Studies conducted in different countries and ecologies show that PGPB significantly increased wheat yield parameters in single applications (Naveed et al., 2008; Zabihi et al., 2011; Hussain et al., 2016; Mukhtar et al., 2017; Sood et al., 2018; Tahir et al., 2018). In addition, it has been reported that biomass, grain yield, macro and micronutrient intake increased significantly compared to the control in studies where different bacteria were administered in multiple combination (Zabihi et al., 2011; Sood et al., 2018). In addition to the yield parameters of wheat, PGPB has been reported to increase the number of germination, increase in germination speed and germination rate (Rana et al., 2011; Laloo et al., 2017), increase the amount of capillary root (Dal Cortivo et al., 2017), increase the root length (Afzal and Bano, 2008; Abbasi et al., 2011; Dal Cortivo et al.,

2017), increase the height of the plant (Afzal and Bano, 2008; Abbasi et al., 2011; Mukhtar et al., 2017) and increase the root and body dry weight (Mukhtar et al., 2017). In addition, plant growth-promoting bacteria containing 1-Aminocyclopropane-1-Carboxylate Deaminase (ACCD), which provides resistance to plant stress conditions, contribute to the growth and development of plants normally by reducing plant ethylene levels under various environmental stress conditions (Çakmakçı, 2009). In the study conducted with Indol Acetic Acid (IAA), siderophore, Hydrocyanic Acid (HCN) and ACC deaminase producing bacteria, germination rate and speed of the bacterium producing siderophore (Rana et al., 2011) was observed to increase. In addition, it has been reported that the bacteria with ACC deaminase activity have a significant increase in the yield parameters (Naveed et al., 2008).

It is aimed to reduce the amount of chemical fertilizers by using PGPB since chemical fertilizers cause significant environmental problems during and after their application to agriculture areas and also due to the use of intensive fossil fuels in their production. For this purpose, it was revealed in the studies conducted on wheat that the use of chemical fertilizers could be reduced by 20% to 50% with the usage PGPBs (Naveed et al., 2008; Zabihi et al., 2011; Abbasi et al., 2011; Hussain et al., 2016; Sood et al., 2018). In a study, it has been reported that PGPBs increase the yield of grain by 30-40% according to chemical phosphorous fertilization (Afzal and Bano, 2008).

Materials and methods

Materials

Wheat species

In this study, Fırat-93 durum and Ceyhan-99 bread wheat varieties were used. Seeds were obtained from Seed Sales Office of General Directorate of Agricultural Enterprises. General characteristics of varieties are below.

Fırat-93: Medium plant height, very waxy and strong stems; narrow, long and steep leaf structure. Spike is medium length, white in color and has black stringiness. It has a fuller, hard and glassy grain and its thousand grain weight is between 45-50 g. It is suitable for summer, middle early and drought tolerant and has good tillering characteristics. It is recommended for rainy, irrigable areas and coastal areas of Southeastern Anatolia. It shows a tolerant reaction to yellow rust disease which can be seen from time to time in the region (Anonymous, 2018a).

Ceyhan-99: Plant height is between 90 and 100 cm. The spikes are white and lengths are medium, and they are upright. It is resistant to bending. Grain color is white, and it has a hard structure. The weight of thousand grains varies between 41 and 45 g. This wheat variety reacts well to fertilizers. Drought and winter resistances are moderate. It is resistant to yellow rust and *Septoria* diseases and it is half resistant to brown rust. Harvest-blending ability is high. The strings are not spilled at harvest time (Anonymous, 2018b).

Bacteria strains

The bacteria were isolated from the Van Lake basin with the TOVAG 108O147 TÜBİTAK project and their PGPB activity was detected (Erman et al., 2010). These bacteria were diagnosed with the microbial identification system (MIS) they were used as double combinations. These bacteria are: *Stenotrophomonas maltophilia* TV14B

(phosphate solubilizing), *Bacillus atrophaeus* TV83D (nitrogen fixation), *Bacillus*-GC group TV119E (phosphate solubilizing), *Cellulomonas turbata* TV54A (nitrogen fixation) and *Bacillus atrophaeus* + *Bacillus*-GC group (TV83D + TV119E)

Methods

Climate and soil properties of the research site

The research was carried out in the laboratory of Field Crops Department of Agricultural Faculty of Siirt University in the period of 2017-2018 and in the trial area of Field Crops Department in dry agricultural conditions. The field trial was established at 37°58'2.18"K and 41°51'13.10"D coordinate point and 538 meters altitude.

In Siirt, terrestrial climate is dominant in general. Summers are hot and dry. The highest air temperature measured is 43.3 °C and the lowest air temperature is -19.5 °C. The average temperature, precipitation and relative humidity values of Siirt, where the research was conducted are presented in *Table 1*. These values cover the long years (1938-2017) as well as the period between October-June 2017-2018. As can be seen from *Table 1*, the 2017-2018 production season was generally dry and the rate of rainfall in March and April decreased. However, the rainfall in the month of May has been 84.6 mm higher than the average of many past years (Anonymous, 2018c). This situation affected the wheat yield negatively.

Table 1. Average climate values of Siirt for long term averages in the past and for 2017-2018 October-June period

Months	Temperature (°C)		Precipitation (mm)		Humidity (%)	
	LTA*	2017-18	LTA*	2017-18	LTA*	2017-18
October	17.9	18.4	49.1	5.4	50.3	34.6
November	10.4	11.2	81.4	85.0	64	64.4
December	4.8	8.0	94.4	48.6	72.4	65.2
January	2.6	5.7	96.8	56.4	72	70.5
February	4.2	8.2	97.5	74.2	66.6	67.7
March	8.3	13.7	111.1	47.6	61.3	55.9
April	13.7	16.8	104.7	61.6	58.2	47.6
May	19.3	19.8	62.0	146.6	49.9	58.9
Jun	26.0	27.4	9.2	3.0	34.1	31.3
Average	11.91	14.36	--	--	58.76	55.12
Total	--	--	706.2	528.4	--	--

*LTA = long-term average (1938-2017)

The soil structure of the trial area where the research is carried out is clayey, has a neutral pH, its lime content is very low, it is salt-free, its K content is sufficient, in terms of organic matter and phosphorus it is poor (*Table 2*).

Table 2. Soil analysis values of the experiment area

pH	Ec s/cm	Lime CaCO ₃ %	Organic matter %	Phosphorus P ₂ O ₅ kg/da	Potassium K ₂ O kg/da	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Sand %	Clay %	Silt %	Texture
6.87	602	0.64	0.90	1.67	114	13.01	1.78	0.60	21.89	41.64	51.32	7.04	L

Trial pattern

The trial consisted of 48 parcels in two separate trials consisting of 24 parcels of 1 × 5 m size with 3 replications, with bread and durum wheat according to the randomized block design.

Fertilizer application

Fertilization has been done to the bacteria untreated parcels as full fertilization with values of 9 kg N/da and 7 kg P₂O₅/da; half of these values were applied in 50% fertilization. In addition, 50% and 100% chemical fertilizers were applied without control and fertilization (0% fertilizer). No bacteria were applied to the parcels where the controls were located. The activity of the bacteria was determined according to the controls. All of the phosphorus and 1/3 of the nitrogen were given during planting and diammonium phosphate (18-46-0) was used. The remaining part of the nitrogen was given by urea fertilizer (46% N) during the period of tillering and staking.

Bacteria application

In the experiment, within the scope of the TÜBİTAK project numbered TOVAG 108O147, nutrient agar (Merck-VM71680604), which was isolated from Van Lake basin and was previously diagnosed with MIS system, was used as a feed-lot for the multiplication of bacteria, PGPB activity of which was exposed to greenhouse and field conditions. 20 g of nutrient agar was added to one liter of distilled water, adjusted to pH 7.0, and the mixture was sterilized by autoclave for 15 min at 121 °C. After sterilization, the feed-lots were cooled to 50 °C, then transferred to petri plates and allowed to solidify. The stock cultures of the bacteria were planted in nutrient agar medium with the help of the needle and incubated at 26 ± 2 °C for 24 h (*Fig. 1*).

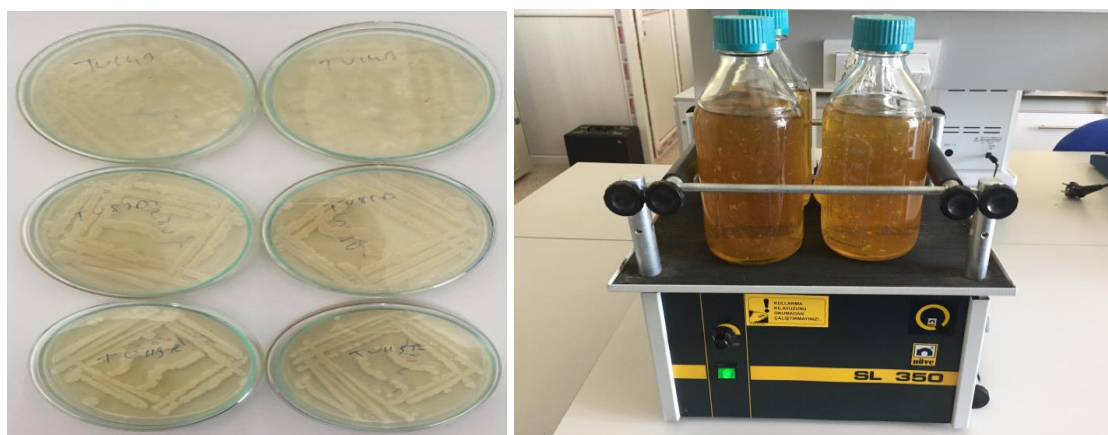


Figure 1. Development of bacteria in nutrient agar and nutrient broth feed-lot

The nutrient broth (Merck-VM775843711) was used as the liquid feed-lot. 8 g of nutrient broth feed-lot was added to one liter of distilled water and pH was adjusted to 7.0. The mixture was sterilized by autoclave for 15 min at 121 °C and then allowed to cool. A single colony was taken from the bacteria developed in nutrient agar feed-lot and was transferred to nutrient broth feed-lot in aseptic conditions. The bacteria transferred to the liquid feed-lot were incubated at 26 ± 2 °C for 24 h and at 120 rpm in

the horizontal shaker. After incubation, the bacteria concentrations were turbidimetrically adjusted to $\sim 10^8$ cfu/ml (Fig. 1). In the final stage, the bacteria were inoculated into the seed. Surface sterilization of the seeds was made with 5% (v/v) sodium hypochlorite for 20 min and washed 3 times with distilled water. Bacteria were applied to the surface sterilized seed for 3 h in order to allow the swelling of the seed (Clark, 1965). Seeds were laid on drying sheets and allowed to dry. Dried seeds were planted on the field one day later. Sterile nutrient broth feed-lot was used as control.

Planting, care, harvest and threshing

The experiment was established on the experimental area of Agricultural Faculty of Siirt University in the 2017-2018 production season with three replications according to the randomized block design (Fig. 2).

The size of the parcels is 5 m, the width is 1 m (5 rows and between each row there is 20 cm) and each parcel area is 5 m². Seeds were planted in parcels as 500 seeds per m². In each parcel, gloves were used to prevent the bacteria from smearing on the seed surface. Experiment was established on 21.11.2017, and the plants sprouted on 01.12.2017 (Fig. 3). Weed control was done by hand. No disease or harmful situation has been observed in the trial area which could cause economic damage.



Figure 2. Land parcelling and planting



Figure 3. Trial and parcel views

With the planting process, diammonium phosphate (DAP) was given as 7 kg phosphorus and 3 kg nitrogen per decare. According to the results of soil analysis, to meet the nutrients needed by plants; in the period of tillering and bolting, urea was applied in the form of upper fertilization with 3 kg of pure nitrogen. 9 kg pure nitrogen and 7 kg phosphorus were given to the plants during planting, tillering and bolting periods. One row on each side of the parcels and 50 cm of space at the beginning and end of the parcel were left and the remaining 2.4 m² area was harvested with the sickle on 01.07.2018. Then it was taken to the warehouse and blended with the machine.

Obtaining data

The experiment was continued during one growing season. Plant height (cm), spike length (cm), number of tillerings (pcs), number of spikes per square meter (pcs/m²), number of spikelets per spike (number/spike), number of grains per spike (pieces/spike), weight of thousand grams (grams), grain yield (kg/da), biological yield (kg/da), hectoliter weight (kg/100 l), harvest index (%) values were determined based on the methods applied in the studies of Tosun et al. (1971) and Ünver (1995).

Statistical evaluation of the results

The mean values obtained from the data were analyzed using the Statix 10 package program. Grouping of means was done according to Tukey's HSD test.

Results

Plant height (cm)

In terms of applications, the highest plant height of Firat-93 durum wheat was obtained from *Stenotrophomonas maltophilia* (TV14B) with 74.13 cm. and the lowest plant height was obtained from non-inoculated and non-fertilized control with 68.23 cm. Plant sizes in other applications are among these two values (Table 3).

Table 3. Average values of plant height in Firat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Firat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	68.23	71.67 ^b
50% fertilized control	70.76	78.20 ^a
100% fertilized control	72.43	77.33 ^a
<i>Stenotrophomonas maltophilia</i> (TV14B)	74.13	80.07 ^a
<i>Bacillus atrophaeus</i> (TV83D)	73.36	78.60 ^a
<i>Bacillus</i> -GC Group (TV119E)	73.71	79.47 ^a
<i>Cellulomonas turbata</i> (TV54A)	72.80	77.50 ^a
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	72.33	78.40 ^a
Average	72.22	77.65
P	0.1002	0.0003
Tukey's HSD	6.4455	4.3347

Mean values in columns shown with different letters are significant at p < 0.05 level according to Tukey's HSD test

The highest plant height of the Ceyhan-99 bread wheat was obtained from the application of *Stenotrophomonas maltophilia* (TV14B) with 80.07 cm. and the lowest plant height was obtained as 71.67 cm. from non-inoculated and non-fertilized control. Plant sizes in other applications are among these two values. Plant height in TV14B bacterial application was observed to be 11% higher than non-inoculated and non-fertilized control. However, in all bacterial applications, the height of the plant was observed to be higher than non-inoculated and non-fertilized control (Table 3).

Spike length

The highest spike length of the Fırat-93 durum wheat was obtained from *Bacillus*-GC Group (TV119E) bacteria with 5.92 cm. and the lowest spike length was obtained from non-inoculated and non-fertilized control as 5.23 cm. Spike length in other applications is among these two values. In addition, it was observed that other bacterial applications had a positive and significant effect on spike size compared to non-inoculated and non-fertilized control (Table 4).

Table 4. Average values of spike size in Fırat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Fırat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	5.23 ^b	6.45 ^c
50% fertilized control	5.38 ^{ab}	6.94 ^{bc}
100% fertilized control	5.42 ^{ab}	6.98 ^{bc}
<i>Stenotrophomonas maltophilia</i> (TV14B)	5.69 ^{ab}	7.90 ^a
<i>Bacillus atrophaeus</i> (TV83D)	5.84 ^{ab}	7.22 ^{ab}
<i>Bacillus</i> -GC Group (TV119E)	5.92 ^a	7.92 ^a
<i>Cellulomonas turbata</i> (TV54A)	5.86 ^{ab}	7.63 ^{ab}
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	5.77 ^{ab}	7.37 ^{ab}
Average	5.64	7.30
P	0.0126	0.0001
Tukey's HSD	0.6471	0.7668

Mean values in columns shown with different letters are significant at $p < 0.05$ level according to Tukey's HSD test

The highest spike length of the Ceyhan-99 bread wheat was obtained from the *Bacillus*-GC Group (TV119E) bacteria with 7.92 cm. The lowest spike length was obtained from non-inoculated and non-fertilized control with 6.45 cm. Spike length in other applications is among these two values. It has been observed that bacterial applications have a positive and statistically significant effect on the increase of spike length compared to all three controls (Table 4).

Tillering count

In terms of applications, the highest number of tillerings of Fırat-93 durum wheat was obtained from 2.93 to 50% fertilized control application, while the lowest number of tillerings was obtained by *Cellulomonas turbata* (TV54A) with 2.52. The number of tillerings in other applications was among these two values. Only *Bacillus atrophaeus*

(TV83D) (2.73 count) bacterial application increased in terms of the number of tillerings compared to non-inoculated and non-fertilized control (2.63 count) and other bacterial applications remained below this value (Table 5).

Table 5. Average values of number of tillerings in Firat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Firat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	2.63	2.07
50% fertilized control	2.93	2.30
100% fertilized control	2.76	3.27
<i>Stenotrophomonas maltophilia</i> (TV14B)	2.57	2.07
<i>Bacillus atrophaeus</i> (TV83D)	2.73	2.23
<i>Bacillus</i> -GC Group (TV119E)	2.60	2.50
<i>Cellulomonas turbata</i> (TV54A)	2.52	2.53
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	2.53	2.27
Average	2.66	2.40
P	0.722	0.2748
Tukey's HSD	0.8756	1.6299

The highest number of tillerings of Ceyhan-99 bread wheat was obtained from the application of 100% fertilized control with 3.7. The lowest number of tillerings was obtained with 2.07 from non-inoculated and non-fertilized control and *Stenotrophomonas maltophilia* application (TV14B). The number of tillerings in other applications was among these two values. With the exception of *Stenotrophomonas maltophilia* (TV14B), there were positive increases in other bacterial applications compared to non-inoculated and non-fertilized control (Table 5).

Number of spikes per square meter

In terms of applications, the highest number of spikes per square meter that belongs to Firat-93 durum wheat was obtained as 450 from 50% fertilized control application. The lowest number of spike number for square meter was obtained from *Cellulomonas turbata* (TV54A) bacteria application with 291.67. In other applications, the number of spikes per square meter was between these two values. It has been found that bacterial applications generally do not contribute to the increase in the number of spikes per square meter compared to the controls, and that the number of spikes per square meter in all bacterial applications is lower than the general average (369.79) (Table 6).

The highest number of spikes per square meter of Ceyhan-99 bread wheat was obtained as 535.33 from 100% fertilized control application, while the lowest number of spikes were obtained as 370.00 from non-inoculated and non-fertilized control bacterial application. In other applications, the number of spikes per square meter was between these two values. It has been observed that bacterial applications have more spike numbers per square meters compared to non-inoculated and non-fertilized control. According to the results of the study, the highest number of spikes per square meter was obtained in chemical fertilizer applications (Table 6).

Table 6. Average values of spike number per square meter in Firat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Firat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	360.00 ^{ab}	370.00
50% fertilized control	450.00 ^a	483.33
100% fertilized control	401.00 ^{ab}	535.33
<i>Stenotrophomonas maltophilia</i> (TV14B)	365.00 ^{ab}	421.67
<i>Bacillus atrophaeus</i> (TV83D)	391.67 ^{ab}	456.67
<i>Bacillus</i> -GC Group (TV119E)	305.00 ^{ab}	396.67
<i>Cellulomonas turbata</i> (TV54A)	291.67 ^b	386.67
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	393.33 ^{ab}	373.33
Average	369.79	427.96
P	0.0331	0.1554
Tukey's HSD	146.67	217.04

Mean values in columns shown with different letters are significant at $p < 0.05$ level according to Tukey's HSD test

Spikelet count of the spike

In terms of applications, the highest number of spikelets that belong to Firat-93 durum wheat was obtained from *Bacillus*-GC Group (TV119E) bacteria application with 18.10 and the lowest number of spikelets was obtained as 15.93% with 50% Fertilized Control application. In other applications, the number of spikelets was between these two values. However, it was observed that *Stenotrophomonas maltophilia* (TV14B) and *Bacillus atrophaeus* (TV83D) bacterial applications did not affect the increase in the number of spikelets (Table 7).

Table 7. Average values of the number of spikelets per spike in Firat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Firat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	16.90 ^{ab}	14.43 ^b
50% fertilized control	15.93 ^b	15.70 ^{ab}
100% fertilized control	16.77 ^b	16.97 ^{ab}
<i>Stenotrophomonas maltophilia</i> (TV14B)	16.53 ^b	16.83 ^{ab}
<i>Bacillus atrophaeus</i> (TV83D)	16.83 ^b	16.93 ^{ab}
<i>Bacillus</i> -GC Group (TV119E)	18.10 ^a	16.87 ^{ab}
<i>Cellulomonas turbata</i> (TV54A)	17.07 ^{ab}	17.57 ^a
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	16.47 ^b	17.23 ^{ab}
Average	16.82	16.56
P	0.0025	0.0356
Tukey's HSD	1.2782	2.8926

Mean values in columns shown with different letters are significant at $p < 0.05$ level according to Tukey's HSD test

In terms of applications, the highest number of spikelets of Ceyhan-99 bread wheat was obtained from *Cellulomonas turbata* (TV54A) as 17.57 and the lowest number of spikelets was obtained from non-inoculated and non-fertilized control as 14.43. In other applications, the number of spikelets in the spike was between these two values (Table 7).

Grain count of the spike

In terms of the applications, the highest number of grains per spike for Fırat-93 durum wheat is 36.40 with *Bacillus*-GC Group (TV119E) bacterial application, the lowest number of grains per spike obtained as 27.93 with 50% Fertilized Control application. The number of grains per spike in other applications was between these two values (Table 8).

Table 8. Average values of the number of grains per spike in Fırat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Fırat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	28.70 ^b	25.63
50% fertilized control	27.93 ^b	29.90
100% fertilized control	29.13 ^b	31.67
<i>Stenotrophomonas maltophilia</i> (TV14B)	29.10 ^b	31.06
<i>Bacillus atrophaeus</i> (TV83D)	29.77 ^{ab}	33.03
<i>Bacillus</i> -GC Group (TV119E)	36.40 ^a	33.63
<i>Cellulomonas turbata</i> (TV54A)	30.87 ^{ab}	33.33
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	30.47 ^{ab}	34.53
Average	30.29	31.60
P	0.0264	0.1686
Tukey's HSD	7.2193	10.615

Mean values in columns shown with different letters are significant at $p < 0.05$ level according to Tukey's HSD test

The highest number of grains per spike of Ceyhan-99 bread wheat is observed to be 34.54 with *Bacillus atrophaeus* + *Bacillus*-GC Group (TV83D + TV119E) bacterial application and the lowest grain number per spike was obtained as 25.63 with non-inoculated and non-fertilized control. The number of grains per spike in other applications was between these two values. The data obtained from all bacterial applications were found to be higher on average than the non-fertilized and non-inoculated and 50% fertilized control applications (Table 8).

Thousand grain weight

In terms of applications, the highest thousand grain weight of Fırat-93 durum wheat is obtained from 100% fertilized control application as 47.99 g. while the lowest thousand grain weight is obtained from *Bacillus atrophaeus* (TV83D) application as 43.88 g. The weight of one thousand grain in other applications was between these two values (Table 9).

Table 9. Average values of thousand grain weight in Firat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Firat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	46.30	38.45
50% fertilized control	46.17	38.81
100% fertilized control	47.99	38.45
<i>Stenotrophomonas maltophilia</i> (TV14B)	45.54	40.03
<i>Bacillus atrophaeus</i> (TV83D)	43.88	38.28
<i>Bacillus</i> -GC Group (TV119E)	45.87	39.30
<i>Cellulomonas turbata</i> (TV54A)	46.46	39.42
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	46.80	39.60
Average	46.13	39.00
P	0.0706	0.0806
Tukey's HSD	3.7015	2.268

The highest thousand grain weight of the Ceyhan-99 bread wheat was obtained from the application of *Stenotrophomonas maltophilia* (TV14B) application as 40.03 g. and the lowest thousand grain weight was obtained as 38.28 g. with *Bacillus atrophaeus* (TV83D) application. One thousand grain weight in other applications is among these two values (Table 9).

Grain yield

In terms of applications, the highest grain yield of Firat-93 durum wheat was obtained from 100% fertilized control application as 288.89 kg/da. The lowest grain yield was obtained from *Bacillus atrophaeus* + *Bacillus*-GC Group (TV83D + TV119E) bacteria application as 218.75 kg/ha.

Table 10. Average values of grain yield in Firat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Firat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	243.06	256.95 ^c
50% fertilized control	256.25	354.17 ^{ab}
100% fertilized control	288.89	360.00 ^a
<i>Stenotrophomonas maltophilia</i> (TV14B)	275.69	304.08 ^{abc}
<i>Bacillus atrophaeus</i> (TV83D)	283.33	319.45 ^{abc}
<i>Bacillus</i> -GC Group (TV119E)	229.17	285.41 ^{abc}
<i>Cellulomonas turbata</i> (TV54A)	244.44	273.61 ^{bc}
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	218.75	274.30 ^{bc}
Average	254.95	303.50
P	0.1174	0.0052
Tukey's HSD	89.116	85.447

Mean values in columns shown with different letters are significant at $p < 0.05$ level according to Tukey's HSD test

The grain yield in other applications was between these two values. In addition, *Bacillus atrophaeus* + *Bacillus*-GC Group (TV83D + TV119E) and *Bacillus*-GC Group (TV119E) bacterial applications were found to be scarcer and less effective on grain yield compared to the non-inoculated and non-fertilized control. However, yield in *Bacillus atrophaeus* (TV83D) (283.33 kg/da) and *Stenotrophomonas maltophilia* bacterial applications (TV14B) (275.69 kg/da) was found to be close to the full dose fertilizer application (288.89) (Table 10).

The highest grain yield of Ceyhan-99 bread wheat was obtained from 100% fertilized control as 360.00 kg/da and the lowest grain yield was obtained from non-inoculated and non-fertilized control application as 256.95 kg/da. The grain yield in other applications was between these two values. Bacterial applications were found to contribute to grain yield compared to non-inoculated and non-fertilized control, but the yield was 50% and 100% lower than fertilized control. It was observed that *Bacillus atrophaeus* (TV83D) bacterial application showed a 24% increase in yield compared to non-inoculated and non-fertilized control (Table 10).

Biological yield

In terms of applications, the highest biological yield of Firat-93 durum wheat was obtained from *Stenotrophomonas maltophilia* (TV14B) bacteria as 756.95 kg/da. The lowest biological yield was obtained from *Bacillus atrophaeus* + *Bacillus* -GC Group (TV83D + TV119E) bacterial application as 565.97 kg/da. Biological yields in other applications are among these two values. *Bacillus*-GC Group (TV119E) and *Cellulomonas turbata* (TV54A) bacterial applications were found to have a negative effect on biological yield. It was observed that *Stenotrophomonas maltophilia* (TV14B) application had the highest biological yield (756.95 kg/da) and was found to be more efficient than the yield in full dose fertilizer application (752.78 kg/da) (Table 11).

Table 11. Average values of biological yield in Firat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Firat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	745.14 ^a	615.28 ^c
50% fertilized control	704.17 ^{ab}	850.00 ^a
100% fertilized control	752.78 ^a	794.00 ^{ab}
<i>Stenotrophomonas maltophilia</i> (TV14B)	756.95 ^a	672.92 ^{bc}
<i>Bacillus atrophaeus</i> (TV83D)	699.66 ^{ab}	704.17 ^{ab}
<i>Bacillus</i> -GC Group (TV119E)	577.78 ^b	653.47 ^{bc}
<i>Cellulomonasturbata</i> (TV54A)	627.78 ^{ab}	655.56 ^{bc}
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	565.97 ^b	654.86 ^{bc}
Average	678.78	700.09
p	0.0017	0.0014
Tukey's HSD	154.21	156.68

Mean values in columns shown with different letters are significant at $p < 0.05$ level according to Tukey's HSD test

The highest biological yield of Ceyhan-99 bread wheat was determined as 850.00 kg/da from 50% fertilized control application and the lowest biological yield was observed as 615.28 kg/da from non-inoculated and non-fertilized control application. Biological yields in other applications are among these two values. Bacterial applications were found to contribute to the increase in biological yield compared to non-inoculated and non-fertilized control (Table 11).

Hectoliter weight

In terms of applications, the highest hectoliter weight of Fırat-93 durum wheat was obtained as 77.60 kg with 50% fertilized control application, the lowest hectoliter weight was obtained as 74.13 kg from *Bacillus atrophaeus* + *Bacillus* -GC Group (TV83D + TV119E) bacterial application, hectoliter weight in other applications were among these two values. At the same time, according to the full dose fertilizer application (76.13 kg), *Stenotrophomonas maltophilia* (TV14B) bacterial application (77.60 kg) was found to be higher but not statistically significant (Table 12).

Table 12. Average values of hectoliter weight in Fırat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains

Application	Fırat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and NON-FERTILIZED CONTROL	76.80	75.33
50% fertilized control	76.00	77.47
100% fertilized control	76.13	74.73
<i>Stenotrophomonas maltophilia</i> (TV14B)	77.60	76.13
<i>Bacillus atrophaeus</i> (TV83D)	75.47	76.53
<i>Bacillus</i> -GC Group (TV119E)	74.13	76.00
<i>Cellulomonas turbata</i> (TV54A)	75.93	76.00
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	74.27	77.73
Average	75.79	76.24
P	0.1276	0.2728
Tukey's HSD	4.14	4.1987

The highest hectoliter weight of the Ceyhan-99 bread wheat was obtained as 77.73 g. with *Bacillus atrophaeus* + *Bacillus* -GC Group (TV83D + TV119E) bacterial application and the lowest hectoliter weight was obtained as 74.13 g. with *Stenotrophomonas maltophilia* (TV14B) bacterial application. The weight of hectoliter in other applications was between these two values. It was observed that the bacterial application with the highest values, *Bacillus atrophaeus* + *Bacillus* -GC Group (TV83D + TV119E) (77.73 kg), was higher than chemical fertilizer applications. In terms of hectoliter weight, which is an important quality parameter, *Bacillus atrophaeus* + *Bacillus*-GC Group (TV83D + TV119E) bacterial application having the highest value suggests that bacterial preparations may be an alternative to chemical fertilizer (Table 12).

Harvest index

In terms of applications, the highest harvest index of Fırat-93 durum wheat was obtained from *Bacillus atrophaeus* (TV83D) bacterial application as 40.16 and the lowest harvest index was obtained from non-inoculated and non-fertilized control application as 32.91. The harvest index in other applications is among these two values. In general, it was observed that all of the bacterial applications caused a statistically positive and significant increase in the harvest index compared to chemical fertilization (Table 13).

Table 13. Average values of harvest index in Fırat-93 durum and Ceyhan-99 bread wheat varieties inoculated with different bacterial strains (%)

Application	Fırat-93 durum wheat	Ceyhan-99 bread wheat
Non-inoculated and non-fertilized control	32.91	41.70
50% fertilized control	36.54	41.79
100% fertilized control	38.48	45.08
<i>Stenotrophomonas maltophilia</i> (TV14B)	36.60	45.02
<i>Bacillus atrophaeus</i> (TV83D)	40.16	45.34
<i>Bacillus</i> -GC Group (TV119E)	39.69	43.66
<i>Cellulomonas turbata</i> (TV54A)	39.25	41.94
<i>Bacillus atrophaeus</i> + <i>Bacillus</i> -GC Group (TV83D+TV119E)	38.71	41.95
Average	37.79	43.30
P	0.1503	0.2919
Tukey's HSD	8.65	7.0067

The highest harvest index of the Ceyhan-99 bread wheat was obtained from *Bacillus atrophaeus* (TV83D) bacterial application as 45.34% and the lowest harvest index was obtained from non-inoculated and non-fertilized control application as 41.70%. The harvest index in other applications is among these two values. The average of bacterial applications was generally higher than non-inoculated and non-fertilized control (Table 13).

Discussion

Considering the environmental hazards of chemical fertilizers, the fact that plant height values, which are known to increase with nitrogenous fertilizers, do not show a significant difference between the bacterial applications, indicates that the bacterial applications show promising effect. In addition to the fact that no bacterial application studies were performed on wheat before, it was stated that bacterial inoculation related to bacterial growth promotes the nitrogen content and vegetative development of the plant and this increases the plant height. (Şahin et al., 2004; Mukhtar et al., 2017; Kumar et al., 2014; Abbasi et al., 2011; Sultana et al., 2016; Arshadullah et al., 2017). Zafar-ul-Hye et al. (2017) reported that the bacterial applications increased the spike size.

In previous studies, it has been shown that inoculating the seeds in the form of double combination application results in significant increases in yield and yield

components compared to control (Darmwal and Gaur, 1988; Rai and Gaur, 1988; Şahin et al., 2004). Sood et al. (2018) reported in their study that bacterial application increased the number of tillerings by 28.3%. As a result of our study, it is thought that differentiation of bacterial strains, wheat varieties, climate and soil characteristics may cause in obtaining different findings. According to the results of our research, the highest number of spikes per square meter was obtained in chemical fertilizer applications. It has also been reported by the researchers that nitrogen contributes to healthy plant and spike formation by increasing vegetative growth in the plant and therefore increases the number of spikes per square meter (Millet and Feldman, 1984; Davidson and Chevalier, 1990; Öztürk et al., 2003; Salantur, 2003). Öztürk et al. (2003) investigated the effect of plant growth-promoting bacteria inoculation on wheat and barley yield at various nitrogen fertilization levels. They found that inoculation with *Bacillus* sp. OSU-142 significantly affected the number of spikelets in wheat spikes but did not have a significant effect on other characters.

It has been reported by many researchers that bacterial inoculation increases the number of grains per spike compared to non-inoculated and non-fertilized control (Öztürk et al., 2003; Salantur, 2003; Sood et al., 2018). In addition, it is said that double and single bacterial inoculation increases the number of grains in the spike (Dokuyucu et al., 1997; Afzal and Bano, 2008; Nain et al., 2010). Bhattarai and Hess (1993) and Das and Saha (2005) reported in their field study that bacterial applications were effective in increasing the thousand grain weight and combined inoculation was more effective than single inoculants. Although there is no similarity between the findings of the cited studies and the findings of the durum wheat variety, some researchers have reported that bacterial applications have a positive effect on the thousand grain weight (Zahir et al., 2009; Mukhtar et al., 2017; Sood et al., 2018). It is thought that this situation is caused by working with different bacteria and varieties.

Many researchers have reported that bacterial inoculation increases grain yield (Barazani and Friedman, 1999; Şahin et al., 2004; Çakmakçı et al., 2005; Çakmakçı et al., 2007; Behera and Rautaray, 2010). In this respect, the results obtained are similar to the previous studies. In previous studies, it has been reported by many researchers that chemical fertilization and bacterial inoculation provide increases in biological yields according to control (Kumar and Ahlawat, 2006; Sultana et al., 2016; Dos Santos et al., 2017; McCarty et al., 2017; Inwati et al., 2018; Sood et al., 2018). Çiğ (2011) reported that bacterial applications do not affect hectoliter. The researcher also stated that the two-bacterial applications cause an increase in the harvest index, but the single bacterial application showed the lowest value.

In terms of the effect of bacteria on varieties; *Bacillus*-GC group TV119E bacteria application in durum wheat on spike size, spikelet count, the grain count on the spike has been found to have a statistically significant positive effect. In bread wheat, it was observed that it has a significant positive effect on spike size. *Stenotrophomonas maltophilia* TV14B (phosphate solubilizing) bacterial application statistically significantly increased the biological yield of durum wheat and plant height in bread wheat varieties. *Bacillus atrophaeus* TV83D (nitrogen-fixation) bacterial application has been found to have a statistically significant effect on the increase of the biological yield of bread wheat. None of the bacterial applications had statistically significant effect on the number of tillerings, weight of thousand grains, hectoliter weight and harvest index in both varieties.

All bacterial applications in the bread wheat varieties increased the grain yield statistically significantly compared to the non-inoculated and non-fertilized control. In particular, bacterial application of *Bacillus atrophaeus* (TV83D) increased yield by 24% compared to non-inoculated and non-fertilized control. This bacterial strain also yielded an increase on yield in durum wheat, but it was not found to be statistically significant compared to other applications. The fact that durum wheat grain yield (283.33 kg/da) is close to 100% chemical fertilization (288.89 kg/da) shows that this bacterium significantly contributes to yield. In addition, the yield of durum wheat varieties in 100% chemical fertilizer (288.89 kg/da) was less than the bread wheat (360.00 kg/da). It is believed that this situation may be caused by the fact that the water demand for durum wheat is higher than that of bread wheat and the drought in 2018 spring months has a negative effect on the development of durum wheat.

As a result, bacterial applications significantly increased the yield in bread wheat compared to non-inoculated and non-fertilized control. In addition, it has been determined that bacterial applications show different reactions according to varieties and different bacteria have different effects on plant yield. The highest grain yield increase among microorganism applications was obtained from *Bacillus atrophaeus* (TV83D) bacterial application. In this respect, it is thought that this bacterial strain can be used as a bio-fertilizer material.

Conclusion

At the end of the study, mainly *Bacillus atrophaeus* (TV83D) which has a positive effect on all parameters, *Bacillus*-GC group TV119E and *Stenotrophomonas maltophilia* TV14B bacteria strains were found to be promising. However, in order to determine the effectiveness of the bacteria used in the study, it is thought that field trials should be established on different plant species and under different ecological conditions, at least for two years. In addition, in later studies, it is thought that testing of bacterial applications and other organic fertilizers (barn, worm etc.) will contribute to revealing the potential of these bacteria.

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