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Original Research

Determination of Yield Parameters of Barley (*Hordeum vulgare* L.) Inoculated with Phosphorous-Solubilizing and Nitrogen-Fixing Bacteria

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Abstract

Chemical fertilizer use efficiencies should be increased to reduce fertilizer quantities and potential negative impacts of excessive uses on soil and environmental health. Therefore, alternative soil nutrient sources are searched for and biological alternatives offer promising outcomes in this sense. In this study, effects of single, dual and triple combinations of phosphorous-solubilizing (*Bacillus megatherium* var. *phosphaticum* [M-13]) and N-fixing bacteria (*Stenotrophomonas maltophilia* [82] and *Ralstonia pickettii* [73]) treatments on plant growth, yield and yield component of barley were investigated and potential effects were compared with chemical fertilizers (N (80 kg ha^{-1} N), P (50 kg ha^{-1} $P_2 0_5$) and N+P (80 kg ha^{-1} + 50 kg ha^{-1} $P_2 0_5$) and Control treatments. The longest grain filling period was obtained from *Ralstonia pickettii, Stenotrophomonas maltophilia* and *Bacillus megatherium* var. *phosphaticum* + *Stenotrophomonas maltophilia* bacteria treatments. The highest number of spikes per m^2, 1000-kernel weight, and test weight values were obtained from sole N treatments, the highest number of kernels per spike, protein content, grain yield, and biological yield from N+P treatments and the highest harvest index values from *Ralstonia pickettii* + *Stenotrophomonas maltophilia* treatments. In general, bacterial treatments resulted in significant changes in yield and yield parameters of barley and significant increases were achieved as compared to the Control.

Keywords: P-solubilizing, N-fixing, bacteria inoculation, yield response, barley

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Introduction

Cereals constitute the largest crop group cultivated worldwide. Throughout the world, almost half of agricultural fields is allocated to cereal farming. In 2022, 147.7 million tons of barley were produced from 51.7 million hectares land area and average yield was 3.50 ton ha^{-1} [1, 2]. In Turkey alone, 7.6 million tons of barley were produced from 2.9 million hectares and average yield was 3.82 ton ha^{-1} [3]. Barley fields of Turkey have prominent arid climate, thus yield is largely designated by amount and distribution of precipitations.

Agricultural fields of Eastern Anatolia Region (Erzurum) are quite poor in organic matter and available phosphorus. In this sense, nitrogen recovery through mineralization of organic matter could not significantly eliminate nitrogen deficiency. Therefore, various fertilizers (chemical fertilizer, biofertilizer) have been used to improve soil fertility. Bacteria inoculations have also been used to improve soil nutrients and availability of existing nutrients. Biofertilizers contain various microorganisms and applied to soils directly or indirectly to make certain essential nutrients available for the plants. Different sources of biofertilizers include nitrogen fixers, phyto-stimulators, phosphate solubilizing bacteria, plant growth promoting rhizobacteria, etc [4]. Application of biofertilizers have become a must to get a high yield and quality and to avoid the environmental pollution [5]. Nitrogenous and phosphorus fertilizers play a great role in plant nutrition [6, 7]. Bio-fertilizers usually contains microorganisms with specific functions such as azospirillum to fix N_2 and P solubilizing bacteria to solubilize P of the soil and fertilizer to be available to the plants [8]. However, phosphorus has a synergistic effect on nitrogen uptake. Phosphorus exists in the soil as phosphates with low solubility (mainly as Ca and Fe phosphates). Bacteria inoculation with phosphorous-solubilizing bacteria such as *Bacillus megatherium* var. *phosphaticum* was reported to play an important role in making phosphorus available for plants [9-11]. Moreover, root-associated bacteria possessing 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity assist plants to withstand biotic and abiotic stresses by decreasing the level of ethylene [12-14]. High fertilizer prices and low-income levels of local farmers significantly limit the use of these fertilizers. It is getting more and more difficult to provide commercial (chemical) fertilizers every year based on non-renewable energy, that is so expensive for all developing countries. Then, microbial fertilizers occupy an important place in sustainable farming systems due to their possitive effects on natural resources and environmental pollution. Therefore, nitrogen-fixing bacteria and bacteria that increase the solubility and accordingly intake of phosphorus and other nutrients are widely used agricultural practices. In Turkey, new approaches are needed in developing agricultural policies for the future. In this sense, subsidized policies for the widespread use of commercial fertilizers should also be considered for organic fertilizers.

The primary objective of the present study is to reduce the dependency on chemical fertilizers in summer barley cultivation, to determine the effect of phosphorous-solubilizing bacteria (*Bacillus megatherium* var. *phosphaticum*) and nitrogen-fixing asymbiotic bacterial strains (*Stenotrophomonas maltophilia* and *Ralstonia picketii*) (both alone and inoculated) on plant growth, yield and yield components of barley under field conditions.

Material and Methods

Location

Experiments were conducted over the experimental fields of Agricultural Research and Extension Center at Agricultural Faculty of Atatürk University. Experiments were conducted for two years and Tokak 157/37 barley genotype was used as the primary seed material of the study.

Climate and Soil Characteristics

Total precipitation and average temperatures in May, June, July, and August were 121.7, 40.7, 2.4, 1.3 mm and 9.7, 14.5, 17.9 and 19.6ºC in 2004; 92.1, 70.0, 20.3, 24.3 mm and 10.6, 13.9, 20.2 and 20.4ºC in 2005, respectively [15]. In terms of total precipitation and average temperatures, 2005 was relatively more favorable for barley cultivation (Table 1). Available phosphorus and potassium values were determined as 22.7-34.3 and 215.8-206.3 kg ha^{-1} for 2004 and 2005, respectively (Table 2). Experimental soils were loamy in texture with an organic matter content of 1.5-1.6% and a pH of 7.6. Available P and K contents were 22.7-34.3 and 215.8-206.3 kg ha^{-1}, respectively.

Design and Treatments

Experiments were conducted in randomized blocks design with 3 replications. A total of 11 treatments were randomly distributed among the plots in each block. There were 33 plots of 6.0 × 1.2 m (7.2 m^2) with 6 plant rows 20 cm apart. A distance of 2 m was left between the blocks and 0.4 m was left between the plots.

Phosphorous-solubilizing bacteria strain of *Bacillus megatherium* and high N-fixing bacteria strains of *Stenotrophomonas maltophilia* (*Xanthomonas, Pseudomonas*) and *Ralstonia pickettii* (*Burkholderia, P. pickettii*) were used as inoculants. Bacteria strains of *Bacillus megaterium* var. *phosphaticum* (M-13), *Stenotrophomonas maltophilia* (82) and *Ralstonia pickettii* (73) were supplied from Plant Protection Department of Atatürk University, Agriculture Faculty. These high N-fixing strains (M-13, 82 and 73) were

Table 1. Climate data for experimental years (LYM, 1990-2005)*.

Parameter	Years	Months				Total & Average
		May	June	July	August	
Monthly total precipitation (mm)	2004	121.7	40.7	2.4	1.3	166.1
	2005	92.1	70.0	20.3	24.3	206.7
	LYM	65.3	40.9	23.4	13.3	142.9
Monthly average temperature (°C)	2004	9.7	14.5	17.9	19.6	15.4
	2005	10.6	13.9	20.2	20.4	16.3
	LYM	10.4	14.5	19.1	19.1	15.8

*Data were supplied from Erzurum Regional Directorate of Meteorology
LYM: 1990-2005, long years' mean

Table 2. Physical and chemical characteristics of experimental soils*.

Years	Texture	Clay (%)	Silt (%)	Sand (%)	pH	Organic matter (%)	Lime (%)	Available	
								P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
2004	Clay-Loam	31.8	40.0	28.2	7.6	1.5	3.1	22.7	215.8
2005	Clay-Loam	31.9	38.9	29.2	7.6	1.6	2.7	34.3	206.3

*Soil analyses were performed in laboratories of soil science department of Atatürk University Agricultural Faculty

Table 3. List of experimental treatments

Control	Control treatment
M-13	*Bacillus megatherium* var. *phosphaticum* (phosphorus-solubilizing bacteria inoculation)
73	*Ralstonia pickettii (Burkholderia, P. pickettii)* (N-fixing asymbiotic strain)
82	*Stenotrophomonas maltophilia* (*Xanthomonas*, *Pseudomonas*) (N-fixing asymbiotic strain)
M-13+73	*Bacillus megatherium* var. *phosphaticum + R. pickettii*
M-13+82	*Bacillus megatherium* var. *phosphaticum + S. maltophilia*
73+82	R. pickettii + S. maltophilia
M-13+73+82	*Bacillus megatherium* var. *phosphaticum + R. pickettii + S. maltophilia*
N	Recommended nitrogen dose, N (80 kg/ha N)
P	Recommended phosphate dose, P (50 kg/ha P_20_5)
N+P	Recommended combined N and P dose, NP (80 kg N/ha + 50 kg P_20_5/ha)

isolated in a previous study from the roots of cereal crops grown in Erzurum and Pasinler Plains [16]. Besides single application of bacteria, dual and triple mixtures were also tested (Table 3). Effects of single and combined bacteria treatments on barley were compared with the Control treatments (no inoculation and fertilization) and recommended doses of chemical fertilizer treatments (Table 3).

Seed Inoculation

Pure cultures were grown in nutrient agar for experiments. A single colony from each strain was transferred to a 50-ml flask, containing nutrient broth (beef extract 1g l^{-1}; yeast extract 2g l^{-1}; peptone 5g l^{-1}; sodium chloride 5g l^{-1} and grown aerobically in flasks overnight, on a rotating shaker (200 rpm) at 25ºC. Bacteria-grown nutrient broth was then diluted with sterile distilled water, containing 0.025% tween 20 to a final concentration of 10^8 CFU ml^{-1}. For treatments, seeds were placed in bacterial suspensions of 10^8 CFU ml^{-1} for 30 minutes before sowing.

Crop Management

Soil was prepared to sowing in the spring and seeds were treated with sugar-water solution with bacteria strains. Manual sowing was paracticed in 525 seed rate m^2 seeding rate. Gloves were used for each plot to prevent contamination. In chemical fertilizer applied plots, all of the phosphorus and half of the nitrogen were applied at sowing and the other half of the nitrogen was applied at tillering period. Manual weed control was practiced during the tillering period. Three irrigations (at sowing, tillering and flowering periods) were practiced. Irrigations were terminated when the soil got saturated to prevent bacteria formation (plots were panned to prevent bacterial contamination).

Plants were harvested at full maturity. Side rows and 50 from top and bottom of each plot were removed and remaining 4 m^2 area was harvested by a sickle. Harvested plants were allowed to dry in the field for 3 days, then threshed by a thresher.

Measurements and Statistical Analysis

Parameters such as grain filling period, spikes per m^2, kernels per spike, plant height, 1000- kernel weight, test weight, grain yield, biological yield, harvest index, leaf area per plant, grain protein concentration, heading period protein concentration and physiological maturity period protein concentration were determined in this study. Leaf area was measured at anthesis with an area meter (CID, Inc. model CI-202). Percentage of N was determined using the Kjeldahl method [17]. Data were subject to analysis of variance using MSTAT-C (1991) software package. Duncan's Multiple Range Test was used to determine the differences among the treatments.

Results and Discussion

There were significant differences between years (except for 1000-kernel weight) and experimental treatments for all characteristics investigated. More favorable climate conditions of the year 2005 increased grain filling period, spikes per m^2, kernels per spike, plant height, grain yield, biological yield, leaf area per plant but decreased test weigh, grain protein concentration, heading period protein concentration, physiological maturity period protein concentration. As average of years, except leaf area per plant, investigated parameters were significantly influenced by treatments (Table 4 and 5). Year x treatment interactions were significant for the most parameters mainly due to different effects of bacteria in 2004 and 2005. Mixed microbial cultures allowed their components to interact with each other synergistically, thus, stimulating each other through physical or biochemical activities.

Grain Filling Period (days)

The grain filling periods were determined as 33.4 days in 2005 and 30.2 days in 2004. The grain filling periods in Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatment were determined as 30.3, 32.2, 32.7, 32.5, 31.5, 32.7, 32.7, 32.2, 31.2, 31.5 and 30.2 days, respectively. The longest grain filling period was seen in 73, 82, 73 + 82 and 82 + M3 treatments and applications, the shortest in Control and N + P treatment (Table 4).

Spikes per m^2

In terms of number of spikes m^2, significant differences were seen between the years and experimental treatments. In 2005, greater number of spikes per m^2 was obtained (519.1 spikes). On the other hand, spikes per m^2 in Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were 387.4, 457.8, 412.2, 398.2, 550.3, 536.3, 436.2, 541.2, 551.8, 434.7 and 480.2 spikes, respectively. The highest number of spikes per m^2 was seen in N (551.8 spikes) and 73+M3 (550.3 spikes), 73 + 82 + M3 (541.2 spikes) and 82+M3 (536.3 spikes) treatments and the the lowest in Control (387.4 spikes) and 82 (398.2 spikes) treatments (Table 4). The increase in the number of spikes per m^2 in bacterial applications in individual and different combinations had the same effect as the N alone. Compared to the Control treatment, N-alone increased number of spikes per m^2 by 42.6%, double mixture (73+M3) by 42.1% and triple bacterial mixture by 39.8%.

Number of Kernels per Spike

As the average of years and treatments, numbers of kernels per spike was determined as 15.9 kernels. Years and treatments had significant effects on number of kernels per spike. Since, the year 2005 had more favourable conditions for plant growth, number of kernels per spike was higher in this year (16.8 kernels). The number of kernels per spike in Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were 14.8, 14.3, 13.6, 16.0, 16.0, 17.4, 15.2, 16.1, 17.7, 15.0 and 19.2 kernels, respectively. The highest number of kernels per spike was obtained from N+P (19.2 spikes) and N (17.7 spikes) treatments and the lowest 73 and 82 bacteria tareatments and Control treatments (Table 4).

Plant Height (cm)

The average plant height was 67.1 cm. Effects of years and experimental tereatments on plant height were found to be significant. Due to higher rainfall, higher plant height was obtained in 2005. Plant height in Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 56.8, 66.4, 70.5, 70.4, 64.9, 68.7, 62.7, 58.2, 77.6, 70.2 and 71.4 cm. The highest plant height was obtained from N and N+P treatments and the lowest from Control treatments (Table 4).

Table 4. Effects of experimental variables on grain filling period (GFP), spikes per m², kernels per spike, plant height, 1000- kernel weight, test weight, grain yield of barley.

Variable	Grain filling period (days)	Spikes per m^2	Kernels per spike	Plant height (cm)	1000- kernel weight (g)	Test weight (kg)	Grain yield (kg ha^{-1})
Years (Y)							
2004	30.2 b	423.9 b	15.1 b	59.4 b	49.8	72.1 a	2408 b
2005	33.4 a	519.1 a	16.8 a	74.8 a	49.8	67.4 b	2899 a
Mean	31.8	471.5	15.9	67.1	49.8	69.8	2654
Treatment (T)							
Control	30.3 b	387.4 c	14.8 def	56.8 f	46.6 e	68.4 c	1909 e
M3	32.2 ab	457.8 bc	14.3 ef	66.4 bcd	48.5 de	68.4 c	2516 cd
73	32.7 a	412.2 bc	13.6 f	70.5 bc	49.9 bcd	70.4 ab	2565 c
82	32.5 a	398.2 c	16.0 cd	70.4 bc	49.0 cd	70.1 abc	2542 cd
73+M3	31.5 ab	550.3 a	16.0 cd	64.9 cd	51.7 ab	69.4 bc	2375 d
82+M3	32.7 a	536.3 a	17.4 bc	68.7 bc	49.1 cd	69.8 abc	2400 cd
73+82	32.7 a	436.2 bc	15.2 de	62.7 de	50.6 abcd	69.8 abc	2893 b
73+82+M3	32.2 ab	541.2 a	16.1 cd	58.2 ef	48.3 de	69.9 abc	2588 c
N	31.2 ab	551.8 a	17.7 b	77.6 a	52.4 a	71.4 a	3067 b
P	31.5 ab	434.7 bc	15.0 def	70.2 bc	51.1 abc	69.5 abc	3041 b
N+P	30.2 b	480.2 ab	19.2 a	71.4 b	50.8 abc	70.2 abc	3297 a
LSD	1.77	70.05	1.43	5.08	2.04	1.65	17.28
F values							
Y	74.99***	73.98***	53.41***	368.94***	0.07	332.28***	323.44***
T	2.17*	12.03***	18.85***	21.07***	10.12***	3.96***	74.71***
Y x T	0.79	6.35***	4.11***	25.98***	3.76***	1.82	6.94***
CV (%)	4.79	9.54	5.76	4.86	2.63	1.52	4.18

The means with the same letter within variable are not significantly different (Duncan's multiple range test $p<0.05$); *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively.

1000-Kernel Weight (g)

As the average of years and treatments, 1000-kernel weight was determined as 49.8 g. Effects of experimental treatments were found to be significant (years were insignificant). Higher values were obtained in 2005 due to higher precipitations. The 1000- kernel weight of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 46.6, 48.5, 49.9, 49.0, 51.7, 49.1, 50.6, 48.3, 52.4, 51.1 and 50.8 g. The highest 1000-kernel weights were obtained from sole nitrogen fertilizer (52.4 g) and 73 + M3 bacterial mixture (51.7 g) and the lowest values from Control treatments (46.6 g) and triple mixture of bacteria (48.3 g) (Table 4). An increase was observed in all treatments.

Test Weight (kg)

As the average of years and treatments, test weight was determined as 69.8 kg. Effects of years on test weight were found to be significant. Test weights of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 68.4, 68.4, 70.4, 70.1, 69.4, 69.8, 69.8, 69.9, 71.4, 69.5 and 70.2 kg. The highest test weight was obtained from sole nitrogen fertilizer treatments (71.4 kg) and the lowest from Control and M3 treatments (Table 4).

Grain Yield (kg ha^{-1})

In terms of grain yield, differences between years and experimental treatments were found to be significant. Average grain yield was determined as 2654 kg ha^{-1}. Due to higher precipitations, grain yield

Table 5. Effects of experimental variables on biological yield, harvest index (HI), leaf area per plant, grain protein concentration, heading period protein concentration, physiological maturity protein concentration of barley.

Variable	Biological yield (kg ha^{-1})	Harvest index (%)	Leaf area per plant (cm^{-2})	Grain protein concentration (%)	Heading period protein concentration (%)	Physiological maturity period protein concentration (%)
Years (Y)						
2004	9908 b	24.4 b	23.6 b	12.57 a	8.69 a	3.44 a
2005	11243 a	25.9 a	33.2 a	10.49 b	6.71 b	2.83 b
Mean	10576	25.1	28.4	11.53	7.70	3.13
Treatment (T)						
Control	9759 d	19.8 d	29.5	11.02 de	5.92 e	2.55 d
M3	9808 cd	26.0 ab	29.3	12.08 c	6.50 de	2.62 d
73	9940 cd	25.8 ab	25.6	10.15 ef	7.58 c	3.47 ab
82	9842 cd	26.0 ab	26.8	11.72 cd	6.75 d	3.10 c
73+M3	10750 bcd	22.1 cd	31.0	10.57 e	7.62 c	3.20 bc
82+M3	9775 d	24.6 bc	28.9	12.52 bc	8.75 ab	3.03 c
73+82	10226 cd	28.4 a	27.3	10.75 e	8.32 b	3.47 ab
73+82+M3	10674 bcd	24.4 bc	29.3	9.65 f	8.47 b	3.08 c
N	11562 b	26.6 ab	29.9	13.13 b	8.85 ab	3.68 a
P	11083 bc	27.4 ab	25.3	10.63 e	6.77 d	2.57 d
N+P	12913 a	25.5 ab	29.9	14.58 a	9.18 a	3.70 a
LSD	114.5	3.09	ns	0.83	0.60	0.30
F values						
Y	54.48***	8.92***	93.44***	249.81***	439.67***	166.23***
T	10.77***	8.99***	1.31	44.67***	48.87***	29.49***
Y x T	2.50*	4.33***	4.93***	0.60	0.81	5.59***
CV (%)	6.95	7.89	14.24	4.63	4.99	6.09

The means with the same letter within variable are not significantly different (Duncan's multiple range test p<0.05); *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively.

was higher in 2005. Grain yield of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 1909, 2516, 2565, 2542, 2375, 2400, 2893, 2588, 3067, 3041 and 3297 kg ha^{-1}. The highest grain yields were obtained from N + P (3297 kg ha^{-1}), treatments and the lowest from the Control (1909 kg ha^{-1}) treatments (Table 4).

Biological Yield (kg ha^{-1})

Effects of years and experimental treatments on biological yield were found to be significant. Average biological yield was measured as 10576 kg ha^{-1}. Higher biological yield was obtained in the 2nd year with higher precipitations. Biological yield of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 9759, 9808, 9940, 9842, 10750, 9775, 10226, 10674, 11562, 11083 and 12913 kg ha^{-1}. As in grain yield, the highest biological yields was obtained from N + P treatments and the lowest from Control treatments (Table 5). Even though the inoculation of microorganisms together and individually increased the plant biological yields, no difference was observed among themselves.

Harvest Index (%)

Average harvest index was calculated as 25.1%. Effects of years on harvest index were not found to be significant. The harvest index values of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively calculated as 19.8, 26.0, 25.8, 26.0, 22.1, 24.6, 28.4, 24.4, 26.6, 27.4 and 25.5%. The highest harvest index was obtained from 73 + 82 (28.4%) treatments and the lowest from Control treatments (Table 5).

Leaf Area per Plant (cm^{-2})

Effects of years on leaf area were found to be significant. Average value was calculated as 28.4 cm^2. In the second year, leaf area also increased due to more favorable climate conditions. Leaf area per plant in Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively calculated as 29.5, 29.3, 25.6, 26.8, 31.0, 28.9, 27.3, 29.3, 29.9,25.3 and 29.9 cm^{-2}. Experimental treatments did not have any significant effects on leaf area (Table 5).

Grain Protein Content (%)

Years and experimental treatments had significant effects on grain protein contents. Average grain protein content was determined as 11.53%. In 2004, when precipitation was low, a higher grain protein content was obtained. Grain protein contents of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively determined as 11.02, 12.08, 10.15, 11.72, 10.57, 12.52, 10.75, 9.65, 13.13, 10.63 and 14.58%. The highest grain protein contents was obtained from N + P (14.58%) treatments and the lowest from 73 + 82 + M3 bacteria treatments (9.65%) (Table 5).

Heading Period Plant Protein Content (%)

Years and experimental treatments had significant effects on heading period plant protein contents. The average protein content in this period was determined as 7.70%. In 2004, when precipitation was low, a higher protein content was obtained. Heading period plant protein contents of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatmens were respectively determined as 5.92, 6.50, 7.58, 6.75, 7.62, 8.75, 8.32, 8.47, 8.85, 6.77 and 9.18%. The highest heading period plant protein content was obtained from sole nitrogen treatments and the lowest from the Control treatments (Table 5). As compared to Control treatments bacteria treatments increased plant protein contents in this period.

Physiological Maturity Period Plant Protein Content (%)

Years and experimental treatments had significant effects on physiological maturity period plant protein contents. Values were higher in 2014 with lower precipitation levels. Physiological maturity period plant protein contents of Control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 2.55, 2.62, 3.47, 3.10, 3.20, 3.03, 3.47, 3.08, 3.68, 2.57 and 3.70 %. The highest values were obtained from N, N + P and triple bacteria treatments and the lowest from Control and P treatments (Table 5).

In this study, effects of bacteria and chemical fertilizer treatments on yield and yield parameters of barley were investigated. Present findings revealed that climate conditions and experimental treatments had significant effects on yield and yield parameters of barley. Experimental treatments increased grain filling period, number of spikes per m^2, number of grains per spike, grain and biological yield, leaf area per plant. However, test weight, grain protein concent and physiological maturity period plant protein concent values decreased with experimental treatments. Year and treatment interactions were generally found to be significant for most measured parameters chiefly due to different effects of bacteria in both growing seasons.

Changes in climate conditions have significant effects on bacterial activity. Low and high temperatures can negatively affect PGPR activity [18]. As a result of PGPR application under low temperature conditions occurring in the vegetation period, lower yield values can be obtained as compared to chemical fertilizers [19]. On the contrary, in periods when the soil temperature is relatively higher [20, 21], it is stated that PGPR applications affect the yield more positively than other alternative applications.

The bacteria tested in the present study may have increased barley growth and yield due to production of indole acetic acid (IAA). This may be due to the increase in the amount of P available in the soil, phosphate mobilization and N fixation. It was stated in previous studies that IAA (indol acetic acid) production abilities may be required for endophytic and rhizospheric bacteria to contribute plant growth-health, IAA production can enable bacteria to interact with plants [22-24]. Akkopru and Ozaktan (2018) reported that there was a significant relationship between level of IAA produced by these bacteria and the increase in yield [25]. Similarly, Chouyia et al. (2020) reported that phosphate-solubilizing bacteria could represent a potential candidate as a bio-fertilizer to increase plant growth as well as P uptake [26]. In addition, Abbas and Noni (2022) indicated that there was an important increase in seed yield due to the increase in N content, which may increase vital processes as a result of increasing absorption [27]. However, dual and triple bacteria combinations had higher yields than single phosphorus mobilizing or N-fixing bacteria treatments [28].

Grain filling period increased with bacterial applications. The highest increases were obtained from *Ralstonia pickettii*, *Stenotrophomonas maltophilia* and *Bacillus megatherium* var. *phosphaticum* + *Stenotrophomonas maltophilia* bacteria treatments. However, the highest values of spikes per m^2, 1000-kernel weight, test weight, number of kernels per spike, protein content, grain yield, and biological yield were obtained from N and P chemical fertilizer treatments. In bacterial applications, although the highest values were not obtained, they had a positive effect on these parameters and higher values were obtained as compared to the Control. Altuner et al. (2022) stated that the grain yield, total yield, number of spikes per square meter, spike length and 1000-kernel weight values increased with *Bacillus megaterium*, *Bacillus subtilis*, *Lactococcus* spp. bacteria treatments [29]. It was stated in previous studies that number of grains per spike differed based on bacterial treatments and number of grains per spike generally increased with bacterial treatments [30, 31].

Although the best results were observed in chemical fertilizer applications in similar studies on different plants, single, double and triple bacteria combinations were found to be effective on grain filling period, number of spike per square meter, 1000-kernel weight, biological yield, grain yield and grain protein ratio. It was also reported that plant protein ratios in flowering and physiological maturity periods are increased [32-34]. Plant height of barley varies mostly based on plant genetics, fertilization, precipitation and soil characteristics. The highest plant height in present study was observed in sole N and N+P treatments. Average nitrogen availability promotes vegetative development and increases plant height.

The 1000-kernel weight value of the barley plant differed depending on the applications. The highest 1000-kernel weight was obtained from sole nitrogen fertilizer and 73 + M3 bacterial treatments and the lowest from the Control treatments. In similar studies, as a result of *Azospirillum* + *Azotobacter* bacteria treatments and N-P treatments, maximum 1000- kernel weight, grain yield, biological yield and harvest index were obtained as compared to the Control treatments and a minimum was obtained from Control treatments [35]. In another study, it was stated that PGPR bacteria (especially *Azospirillum*) significantly increased grain yield, therefore it could be used instead of chemical fertilizers in sustainable agricultural systems [36-40]. Use of nitrogen-fixing bacteria *Azotobacter* and *Azospirillum* with 100 kg urea decreased nitrogen fertilizer demand up to 50% and increased 1000-kernel weight, nitrogen content and yield of barley [34].

In similar studies examining the effects of bacterial applications on number of spikes per m^2, effects of interactions on number of spikes per m^2 were found to be highly significant [36, 19]. Ozturk et al. (2003) found that the highest number of spikes per m^2 in Tokak 157/37 barley cultivar was obtained from 80 kg ha^{-1} N application together with *Bacillus* and *Azospirillum* [20]. Similarly in this study, the increase in number of spikes per m^2 in bacterial applications in individual and different combinations had the same effect as the sole N treatments.

Additionally, increasing of 1000-kernel weight in barley with PGPR priming was reported by [31]. Several of the isolated strains (*Advenella mimigardefordensis*, *Bacillus cereus*, *Bacillus megaterium* and *Burkholderia fungorum*) were able to significantly improve levels of assimilated phosphate, dry weight of ears and total starch accumulated on ears as compared to non-inoculated plants. Since these strains were able to increase the growth and productivity of barley crops, they could be potentially used as biofertilizers [40]. Endophyte populations of the inoculated bacteria were observed in plants growing under field conditions. The results demonstrated that inoculation of with *Enterobacter ludwigii* was a promising option to increase P levels in plants and could be a technique for application in agricultural industry [39].

Moreover, in both years, inoculation significantly increased P and N content and uptake [41]. Inoculation of wheat with plant growth promoting bacteria increased straw yield [42, 43]. Bacterial inoculation results in profound increment of root surface area by eightfold as compared to uninoculated in barley [24]. Similarly, N-fixing rhizobacteria had a positive effect on above-ground biomass of maize and barley [44, 27, 45]. In both years, N and P treatments increased grain N content, physiological maturity N content, straw yield; sole N applications had the highest grain and plant N contents. The lowest values for majority of the investigated plant parameters were obtained from the Control treatments. Among the bacteria treatments, 73+82 bacteria and triple combination had the highest values for all plant parameters. Outcomes of bacteria treatments were closer to the values of chemical fertilizer applications. Supporting present findings, in a study conducted in Argentina, it was concluded that the application of bacteria without fertilizer treatment had the same biological yield (3.795 kg ha^{-1}) and with the maximum dose of chemical fertilizer applied, a yield increase in 1000-kernel weight in barley was recorded [39]. Moreover, 73 + 82 bacteria and triple combination had higher values for grain N content, physiological maturity N content than the sole P treatments. As compared to the Control treatment, grain N contents, N contents in physiological maturity period, straw yields increased with dual (73 + 82) bacteria inoculation. Dual N-fixating 73 + 82 bacteria combination was followed by triple combination and grain N, straw yield and physiological maturity N content in triple combination were not significantly different from dual bacteria treatments. As compared to the Control treatment, increases were observed in grain N contents, N contents in physiological maturity period, straw yields with triple (73 + 82 + M3) bacteria inoculation. In another research, the highest grain protein content was recorded in nitrogen-fixing bacteria (*Azotobacter chroococcum*) treatments combined with *Bacillus subtilis* and *Pseudomonas fluorescens* [46]. It was reported in a previous study that dry matter production of paddy and barley increased significantly by 10-20% with bacteria inoculations as compared to non-inoculated control [47].

Conclusions

This study was conducted with different bacteria and chemical fertilizer treatments and significant changes were encountered in yield and yield parameters of barley plant. In general, PGPR treatments (*Ralstonia pickettii*, *Stenotrophomonas maltophilia* and *Bacillus megatherium* var. *phosphaticum* + *Stenotrophomonas maltophilia*) significantly increased yield parameters as compared to Control. Although the highest increases in some parameters were obtained from N+P chemical fertilizer treatments, bacterial treatments increased the yield parameters at similar rates with the chemical fertilizer treatments. Thus, relevant bacteria could be considered as a suitable substitute or supplement of chemical phosphorous fertilizers in agricultural systems in both normal and poor soils and has great potential to be developed as a bio-fertilizer which could enhance soil fertility and minimize chemical fertilization. It was concluded based on present findings that these bacteria, which have nitrogen and phosphorous-solubilizing properties, should be used in sustainable agriculture to improve yield and yield parameters of barley.

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Conflict of Interest

The authors declare no conflict of interest.

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