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Determination of Efficiency Parameters of Barley Inoculated with Phosphorous-solubilizing and Nitrogen-fixing Bacteria

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Abstract

In the present study, effects of single, dual and triple combinations of phosphorus-solubilizing (*Bacillus megatherium var. phosphaticum* [M-13]) and N-fixing bacteria (*Stenotrophomonas maltophilia* [82] and *Ralstonia pickettii* [73]) treatments were compared with chemical fertilizer and control treatments with regard to barley efficiency parameters. Efficiency parameters of dual bacteria inoculation treatments yielded closer outcomes to chemical fertilizers and as much as 69.9% N uptake efficiency, 92.5% N translocation efficiency, 70.7% N use efficiency, 87.9% agronomic efficiency, 87.4% physiological efficiency, 87.9% water use efficiencies for grain and 78.7% water use efficiency for biomass of chemical fertilizers (N + P recommended dose) were achieved with dual (73 + 82) bacteria treatments. Nitrogen-fixing dual bacteria treatment (73 + 82) was followed by triple bacteria combination (73 + 82 + M3). Combined bacteria treatments offered environmental friendly practices for soil enhancement and improved yield levels in organic barley farming. Therefore, combined bacterial applications (73 + 82 and 73 + 82 + M3) can be recommended as an alternative fertilization method in organic barley farming.

Keywords P-solubilizing · N-fixing · Bacteria inoculation · Efficiency · Barley · Organic farming

Introduction

Most of the nitrogen and phosphorus applied to the soil is not taken up by plants (Agren et al. 2012). Nitrogen efficiency rate was reported as between 29–42% for cereals (Raun and Johnson 1999). Besides positive effects on crop yields, chemical fertilizers result in environmental pollution. Therefore, alternatives are looked for instead of chemical fertilizers and biological alternatives are yielding promising outcomes. Biological N fixation may constitute a significant alternative in organic barley farming.

Cereals maintain their place in the world as the largest product group in terms of cultivation area and production quantity. Almost half of the cultivated land in the world is allocated to cereal farming. In 2021, barley was grown on a total area of 51.7 million hectares in the world and a total of 159.7 million tons of barley was produced annually (with an average yield of 3.1 tons per hectare) (USDA 2021). In Turkey, annually 5 million 750 thousand ton barley were produced from 3 million 169 thousand hectares (with an average yield of 1.8 tons per hectare) (TUIK 2021). Yield levels are below the world average. Barley farming is practiced under rain-fed conditions in Turkey. Therefore, amount and distribution of precipitations designate yield levels.

Climate and soil fertility designate the yield levels of a region. Soils, which has been maintained without fertilization for years and on which monoculture agriculture has been widely applied, may lose fertility and productivity. Since Eastern Anatolian soils are poor in organic matter, N acquisition through the mineralization of organic matter is not of much importance in elimination of N deficiency. Fertilization is the most important way to address the nutrient deficiency and increase productivity of the soils with low organic matter content and. Bacteria inoculation

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may also offer an effective tool to improve soil productivity (Bhardwaj et al. 2014; Pontes et al. 2015; Singh et al. 2020). Phosphorus is the second most important plant nutrient after N to increase soil productivity and crop production. Phosphorus is major sedentary food element in the soil and its intake by plants occurs more slowly and in smaller quantities (Shen et al. 2011; Namli et al. 2017). However, phosphorus has a synergistic effect on N uptake. Phosphorus is found in soil in the form of less water-soluble phosphates (mainly Ca and Fe phosphates). It is reported that inoculation with phosphorus solvent bacteria such as phosphate played an important role in making phosphorus available for plants (Singh et al. 2020).

Microbial fertilization occupies an important place in sustainable agricultural systems since they may reduce deterioration of natural resources and environmental pollution (Bhardwaj et al. 2014; Alaylar et al. 2021). Therefore, symbiotic and a symbiotic N-fixing bacteria and bacteria that increase the resolution and related intake of phosphorus and the other nutrient elements have been widely used (Rashid et al. 2016; Ju 2018; Singh et al. 2020). New ideas and approaches are needed for formation of future agricultural policies of Turkey. In this sense, State supports should be provided for widespread use of commercial fertilizers and organic fertilizers with positive effects on soil and environment.

This research was conducted to reduce the dependence on chemical fertilizers in spring barley cultivation of Eastern Anatolia Region. Nitrogen uptake and utilization efficiency parameters of phosphorus-dissolving (*Bacillus megatherium var. phosphaticum*) and N-fixing a symbiotic (*Stenotrophomonas maltophilia* and *Ralstonia picketii*) bacterial strains were investigated by field experiments. Chemical fertilizer and control treatments were compared with the single and combined bacteria inoculations.

Materials and Methods

Location

The study was conducted over the experimental fields of Agricultural Research and Extension Center of Atatürk University, Faculty of Agriculture. 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

Average temperatures in May, June, July and August were respectively measured as 9.7, 14.5, 17.9 and 19.6 °C in the first year and as 10.6, 13.9, 20.2 and 20.4 °C in the second year. Average precipitations were respectively measured as 121.7, 40.7, 2.4 and 1.3 mm in the first year and as 92.1, 70.0, 20.3 and 24.3 mm in the second year (Anonymous 2005). In terms of total precipitation and average temperatures, second year was relatively more favorable for barley cultivation. Organic matter content of experiment soils varied between 1.5–1.6% and average pH was determined as 7.6. Available phosphorus contents varied between 22.7–34.3 kg/ha and potassium contents between 215.8–206.3 kg/ha during 2004 and 2005 were respectively. Soils of the experimental fields were slightly alkaline with a clay-loam texture, low organic material and lime, moderate phosphorus, and high potassium contents (therefore no potassium was applied).

Design and Treatments

The experimental design was a randomized block 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.

Table 1 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 $P_2 0_5$)
N + P	recommended combined N and P dose, NP (80 kg N/ha + 50 kg $P_2 0_5$/ha)

Phosphorus-solubilizing bacteria strain of ***Bacillus megatherium*** and high N-fixing bacateria strains of ***Stenotrophomonas maltophilia*** (*Xanthomonas, Pseudomonas*) and ***Ralstonia pickettii*** (*Burkholderia, P. pickettii*) were used as inoculants. Bacateria strains *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 isolated in a previous study from the roots of cereal crops grown in Erzurum and Pasinler Plains (Salantur 2003). Besides single application of bacteria, dual and triple mixtures were also tested (Table 1). 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 1).

Seed Inoculation

The bacteria species and strains were grown in nutrient agar. A single colony from each species and strain was sub-cultured in 50-ml flask containing nutrient broth (yeast extract of 2 g l^{-1}; beef extract of 1 g l^{-1}; sodium chloride of 5 g l^{-1}; peptone of 5 g l^{-1}), grown under aerobic conditions overnight on a rotating shaker (200 rpm) at 25 °C. The sub-cultured bacteria on nutrient broth was then diluted with sterile distilled water, containing 0.025% tween 20 to a final concentration of 10^8 CFU ml^{-1}. Barley seeds were inoculated with bacterial suspensions of 108 CFU ml^{-1} for 30 min before planting.

Crop Management

Soil preparations were conducted in spring when the soil moisture was available for soil tillage. Reproduced high N-fixing bacteria were inoculated to the seeds with the help of sugar water and the seeds were sown manually at a rate of 525 seeds per square meter. To prevent potential contamination, gloves were changed for each bacteria treatment. In the plots where chemical fertilizers were applied, all of the phosphorus and half of the N were applied during the sowing and the other half of the N was applied during the tillering stage. Manual weed control was practiced in all plots. All plots were irrigated three times, once at the beginning of stem elongation, once at the beginning of heading, and the last in the middle of milky ripe periods to saturate the soil and consequently prevent bacteria formation (plots were panned to prevent bacterial contamination).

Plants were harvested at full maturity. Side rows and 0.5 m sections from the top and bottom of each plot were omitted as to consider side effects and harvest was practiced from the remaining 4 m^2 of each plot. Harvested plants were allowed to dry in the field for 3 days, then threshed by a machine.

Measurements and Statistical Analysis

The percentage of N was determined by using the Kjeldahl method in accordance with AACC (1983). N-agronomic efficiency (kg grain/kg N applied), N-uptake efficiency (kg total N uptake/kg N applied), N-utilization efficiency (kg grain/kg total N uptake) and N-translocation efficiency [kg grain N/(kg grain N + kg straw N) × 100] were calculated as described by Anderson (1985), Doyle and Holford (1993), Gonzales Ponce and Salas (1993). Biomass-based water use efficiency (WUEb) was estimated as the ratio of biomass to precipitation between September and August. Grain-based water use efficiency (WUEg) was estimated as the ratio of grain yield to precipitation during the same period as described by Gomez-Macpherson and Richards (1995). Growing degree days were determined using 0 °C as the base temperature. Experimental data were subjected to analysis of variance with the use of MSTAT-C (1991) software and significant means were compared with the use of Duncan's Multiple Range Test (P = 0.01).

Results

Analysis of variance showed significant variations in investigated parameters (except for N use efficiency and grain N yield) of the years. More favorable climate conditions of the second year increased grain yield (data not shown) and straw yield, but decreased grain and straw protein concentration, total N yield and N uptake efficiency. As the average of years, investigated plant traits were all significantly affected by experimental treatments (Table 2 and 3). Except for grain N%, total N yield and N uptake efficiency, year × treatment interactions were significant for other parameters due to different bacterial effects in 2004 and 2005.

In terms of grain N content, variations between the years and between the treatments were significant. Grain N content in 2004 was 2.19%, and it was 1.82% in 2005. Grain N content was higher in the first year due to higher precipitations. Grain N contents of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively identified as 1.68, 1.77, 1.92, 1.87, 1.84, 1.85, 2.10, 2.18, 2.29, 2.04 and 2.54%. The highest grain N contents were obtained from N and N + P treatments and the lowest from the control treatments (Table 2). As compared to the control treatment, 5.4, 14.3, 11.3, 9.5, 10.1, 25.0, 29.8, 36.3, 21.4 and 51.2% higher grain N contents were obtained from M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

Table 2 Effects of bacteria treatments on grain N, physiological maturity N, straw yield, grain N yield, total N yield and N uptake efficiency of barley

Variable	Grain N (%)	Physiological maturity N (%)	Straw yield (kg ha^{-1})	Grain N yield (kg ha^{-1})	Total N yield (kg ha^{-1})	N uptake efficiency (NUpE) (%)
Years (Y)						
2004	2.19 a	0.597 a	7500 b	52.7	97.3 a	42.10 a
2005	1.82 b	0.492 b	8344 a	52.5	93.7 b	26.41 b
Mean	2.00	0.544	7922	52.6	95.5	34.25
Treatment (T)						
Control	1.68 f	0.442 d	7292 b	36.3 d	70.6 d	–
M3	1.77 ef	0.455 d	7300 b	52.6 b	86.4 c	19.70 e
73	1.92 de	0.602 ab	7375 b	44.9 c	89.2 bc	23.24 de
82	1.87 e	0.542 c	7850 b	51.0 b	90.9 bc	25.29 cde
73 + M3	1.84 e	0.558 bc	8375 b	43.3 c	96.3 b	32.13 cd
82 + M3	1.85 e	0.528 c	7375 b	51.9 b	91.0 bc	25.47 cde
73 + 82	2.10 c	0.600 ab	7333 b	53.7 b	97.2 b	33.33 c
73 + 82 + M3	2.18 bc	0.535 c	8087 b	54.6 b	97.0 b	33.05 c
N	2.29 b	0.640 a	8495 ab	69.5 a	116.0 a	56.82 a
P	2.04 cd	0.442 d	8042 b	55.5 b	107.3 a	45.81 b
N+P	2.54 a	0.643 a	9617 a	65.5 a	108.8 a	47.69 b
LSD	0.15	0.05	1131	5.5	8.7	8.96
F values						
Y	250.35***	163.10***	22.31***	0.03	6.88*	112.80***
T	45.51***	29.64***	5.89***	42.78***	29.23***	26.91***
Y × T	0.59	5.47***	3.06**	2.56*	1.64	1.57
CV (%)	4.63	6.13	9.16	6.67	5.86	16.70

The means indicated with the same letters in a column are not significantly different according to Duncan's multiple range test $p < 0.05$; *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively

For N content at physiological maturity period, differences between the years and between the treatments were found to be significant. N content was 0.597% in the first year and 0.492% in the second year. The N content in the second year was lower due to higher precipitation. Nitrogen content at physiological maturity period of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 0.442, 0.455, 0.602, 0.542, 0.558, 0.528, 0.600, 0.535, 0.640, 0.442 and 0.643%. The highest N contents were observed in N + P, N, 73 and 73 + 82 treatments and the lowest N contents were obtained from control, P and M3 treatments (Table 2). As compared to the control, 2.9, 36.2, 22.6, 26.2, 19.5, 35.7, 21.0, 44.8, 0.0 and 45.5% higher N contents were observed in M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

In terms of straw yield, variations between the years and between the treatments were found to be significant. The highest yield was observed in the second year due to higher precipitations. Straw yields in the control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 7292, 7300, 7375, 7850, 8375, 7375, 7333, 8087, 8495, 8042 and 9617 kg ha^{-1}. While the highest values were obtained from N + P and N treatments, the lowest value was obtained from the control treatment (Table 2). As compared to the control treatment, 0.1, 1.1, 7.7, 14.9, 1.1, 0.6, 10.9, 16.5, 10.3 and 31.9% higher straw yields were observed in M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

Grain N yield variations between the years were not significant. Grain N yield was measured as 52.7 kg ha^{-1} in the first year and 52.5 kg ha^{-1} in the second year. Grain N yield variations between treatments were found to be significant. Grain N yields in control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 36.3, 52.6, 44.9, 51.0, 43.3, 51.9, 53.7, 54.6, 69.5, 55.5 and 65.5 kg ha^{-1}. While the highest grain N yields were obtained for N + P and N treatments, the lowest value was obtained from the control treatment (Table 2). As compared to the control treatment, 44.9, 23.7, 40.5, 19.3, 43.0, 47.9, 50.4, 91.5, 52.9 and 80.4% higher grain N yields were obtained from M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

In terms of plant total N yield, variations between the years and between the treatments were found to be signif-

icant. Plant total N yield was 97.3 kg ha^{-1} in the first year and 93.7 kg ha^{-1} in the second year. The lowest plant total N yield was observed in the second year due to higher precipitations. Plant total N yields of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 70.6, 86.4, 89.2, 90.9, 96.3, 91.0, 97.2, 97.0, 116.0, 107.3 and 108.8 kg ha^{-1}. While the highest plant total N yields were obtained from N, N + P and P treatments, the lowest plant total N yield was obtained from the control treatment (Table 2). As compared to the control treatment, 22.4, 26.3, 28.8, 36.4, 28.9, 37.7, 37.4, 64.3, 52.0 and 54.1% higher plant total N yields were obtained in M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

For N uptake efficiency values, differences between the years and between the treatments were found to be significant. N uptake efficiency was identified as 42.10% in the first year and 26.41% in the second year. Lower N uptake efficiency was observed in the second year due to higher temperatures. Nitrogen uptake efficiencies of M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 19.70, 23.24, 25.29, 32.13, 25.47, 33.33, 33.05, 56.82, 45.81 and 47.69%, respectively. While the highest N uptake efficiencies were observed in single N, N + P and single P treatments, respectively, the lowest value was obtained from the single M3 bacteria treatment (Table 2).

For nitrogen translocation efficiency, variations between the years were significant and average of years was 55.1%. However, differences between treatments were not found to be significant. Nitrogen translocation efficiencies of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 45.0, 50.4, 51.8, 55.3, 56.9, 57.2, 56.4, 60.2, 59.8, 52.0 and 61.0%. While the highest N translocation efficiency was observed in N + P treatment, the lowest value was obtained from the control and single M3 bacteria treatments (Table 3). As compared to the control, 12.0, 15.1, 22.9, 26.4, 27.1, 25.3, 33.8, 32.9, 15.6 and 35.6% higher N translocation efficiencies were obtained from M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

For N use efficiency and average N use efficiency, differences between the years were insignificant. Effects of treatments on N use efficiency were significant. Nitrogen use efficiencies of M3, 73, 82, 73 + M3, 82 + M3, 73 + 82,

Table 3 Effects of bacteria treatments on N translocation efficiency, N use efficiency, agronomic efficiency, physiological efficiency, water use efficiency for grain and biomass yield of barley

Variable	N translocation efficiency (NTE) (%)	N use efficiency (NUsE) (kg grain/kg N)	Agronomic efficiency (AE)	Physiological efficiency (PE)	Water use efficiency for grain (WUEg)	Water use efficiency for biomass (WUEb)
Years (Y)						
2004	54.0 b	10.1	30.1 b	24.8 b	14.5 b	59.6 a
2005	56.2 a	10.4	36.3 a	31.2 a	17.5 a	54.4 b
Mean	55.1	10.3	33.2	28.0	16.0	57.0
Treatment (T)						
Control	45.0 e	–	23.9 g	23.8 e	11.5 e	52.1 d
M3	50.4 d	7.6 def	31.5 def	29.2 bc	15.1 cd	52.9 cd
73	51.8 cd	8.3 de	32.1 de	28.9 bc	15.4 c	53.8 cd
82	55.3 bcd	7.9 def	31.8 def	28.6 bc	15.3 cd	53.4 cd
73 + M3	56.9 abc	5.9 f	29.7 f	24.7 de	14.3 d	57.8 bcd
82 + M3	57.2 abc	6.2 ef	30.0 ef	26.4 cde	14.5 cd	52.8 cd
73 + 82	56.4 abc	12.3 c	36.2 c	29.9 b	17.4 b	55.1 bcd
73 + 82 + M3	60.2 ab	8.5 d	32.4 d	27.3 bcd	15.6 c	57.6 bcd
N	59.8 ab	14.5 b	38.3 b	26.5 cde	18.5 b	61.9 b
P	52.0 cd	14.2 bc	38.0 bc	28.5 bc	18.3 b	59.9 bc
N + P	61.0 a	17.4 a	41.2 a	34.2 a	19.8 a	70.0 a
Lsd	4.95	2.01	2.16	2.92	1.04	6.34
F values						
Y	7.48**	0.96	322.75***	189.05***	323.64***	27.47***
T	13.98***	57.50***	74.58***	13.51***	74.73***	10.39***
Y × T	5.63***	8.98***	6.94***	5.08***	6.95***	2.51*
CV (%)	5.77	12.52	4.19	6.69	4.18	7.13

The means indicated with the same letters in a column are not significantly different according to Duncan's multiple range test $p < 0.05$; *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively

73 + 82 + M3, N, P and N + P treatments were respectively identified as 7.6, 8.3, 7.9, 5.9, 6.2, 12.3, 8.5, 14.5, 14.2 and 17.4 kg grain kg^{-1} N. While the highest N efficiencies were obtained from N + P and N treatments, the lowest value was obtained from 73 + M3 bacteria treatment (Table 3).

Effects of years and treatments on agronomic efficiency were both found to be significant. Again, higher agronomic efficiencies were observed in the second year due to higher precipitations. Agronomic efficiencies of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 23.9, 31.5, 32.1, 31.8, 29.7, 30.0, 36.232.4, 38.3, 38.0 and 41.2. While the highest agronomic efficiencies were obtained from N + P and N treatments, the lowest values were obtained from the control and 73 + M3 bacteria treatments (Table 3). As compared to the control, 31.8, 34.3, 33.1, 24.3, 25.5, 51.5, 35.6, 60.3, 59.0 and 72.4% higher agronomic efficiencies were observed in M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

Effects of years and treatments on physiological efficiency were found to be significant and highest values (31.2) were observed in the second year due to higher precipitations. The physiological efficiencies of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 23.8, 29.2, 28.9, 28.6, 24.7, 26.4, 29.9, 27.3, 26.5, 28.5 and 34.2. The highest physiological efficiency was obtained from N + P treatments and the lowest values were obtained from 73 + 82 + M3 bacteria treatments (Table 3). As compared to the control, 22.7, 21.4, 20.2, 3.8, 10.9, 26.6, 14.7, 11.3, 19.7 and 43.7% higher physiological efficiency were obtained from M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

Effects of years and treatments on grain water use efficiency were found to be significant and average of years was 16.0. The grain water use efficiencies of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 11.5, 15.1, 15.4, 15.3, 14.3, 14.5, 17.4, 15.6, 18.5, 18.3 and 19.8. While the highest grain water use efficiency was observed in N + P and N fertilizer treatments, the lowest value was obtained from the control treatment (Table 3). As compared to the control, 31.3, 33.9, 33.0, 24.3, 26.1, 51.3, 35.7, 60.9, 59.1 and 72.2% higher grain water use efficiencies were observed in M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

Effects of years and treatments on plant water use efficiency were found to be significant and average of years was 57.0. The plant water use efficiency values were higher in the first year than in the second year. Plant water use efficiencies of control, M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments were respectively measured as 52.1, 52.9, 53.8, 53.4, 57.8, 52.8, 55.1, 57.6, 61.9, 59.9 and 70.0. While the highest plant water use efficiency was obtained from N + P fertilizer treatments, the lowest plant water use efficiency was obtained from the control treatment (Table 3). As compared to the control, 1.5, 3.3, 2.5, 10.9, 1.3, 5.8, 10.6, 18.8, 15.0 and 34.4% higher plant water use efficiencies for biomass were obtained from M3, 73, 82, 73 + M3, 82 + M3, 73 + 82, 73 + 82 + M3, N, P and N + P treatments, respectively.

Discussion

Results of variance analysis showed that the measured plant parameters were significantly varied between the years (except for grain N yield and N use efficiency) and between the treatments. Favorable climate conditions of the second year increased straw yield, N translocation efficiency, agronomic efficiency, physiological efficiency and water use efficiency for grain, but decreased grain N, physiological maturity N, total N yield, N uptake efficiency and water use efficiency for biomass yield. The best results were achieved in the second year due to both the better climatic conditions this year and the positive effects of the previous year's practices on the soil (Namli et al. 2017, 2021). As the average of years, all of the investigated parameters were significantly affected by the treatments (Tables 2 and 3). Year × treatment interactions were significant for most of the investigated plant parameters (except for grain N, total N yield and N uptake efficiency) chiefly due to different effects of bacteria in both growing seasons.

All of the investigated parameters increased significantly with the bacteria inoculations (except for grain N and physiological maturity N with phosphorus resolvent M3 bacteria inoculation). The bacteria tested in the present study may have increased barley growth and yield due to production of indole acetic acid (IAA), increased amount of root surface area. This may be due to the increase in the amount of P available in the soil, phosphate mobilization and N fixation. The other researchers have stated 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 (Etesami et al. 2015; Babier and Akköprü 2020; Gang et al. 2021). Akköprü and Özaktan 2018 reported that there was a significant relationship between level of IAA produced by these bacteria and the increase in yield. 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. 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. However, dual and triple bacteria combinations had higher yields than single phosphorus mobilizing or N-fixing bacteria treatments (Emami et al. 2018). In a previous study conducted with lettuce in Morocco, the amended compost, PGPR bacteria (BS14 and BS36) and AMF applied alone or in different combinations increased the yield and growth parameters (soluble sugars content, protein content and polyphenol content of lettuce leaves) and gave similar results to those obtained by the use of NPK based fertilizers (Tahiri et al. 2022). Moreover, in both years in barley grains, the co-inoculation significantly increased P and N content and uptake (Espidkar et al. 2017). Inoculation of wheat with plant growth promoting bacteria increased straw yield (Stanojković et al. 2012; Hafez et al. 2021). Bacterial inoculation results in profound increment of root surface area by eightfold as compared to uninoculated in barley (Gang et al. 2021) and wheat (Emami et al. 2018). Similarly, N-fixing rhizobacteria had a positive effect on above-ground biomass of maize and barley (Tang et al. 2020; Abbas and Noni 2022; Liu et al. 2022), below ground biomass of wheat (Zhu et al. 2014) and positive effect on quality parameters of tomato (Yagmur and Gunes 2021; El-Beltagi et al. 2022).

In both years, N and P treatments increased grain N content, physiological maturity N content, straw yield, N translocation efficiency, N use efficiency, agronomic efficiency, water use efficiency for grain and water use efficiency for biomass; single N applications had the highest grain N yield, total N yield, N uptake efficiency. 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 (3795 kg/ha) and with the maximum dose of chemical fertilizer applied a yield increase in thousand seed weight in barley was recorded (Zaballa et al. 2020). Moreover, 73 + 82 bacteria and triple combination had higher values for grain N content, physiological maturity N and N translocation efficiency parameters than the single P treatment. As compared to the control treatment, grain N contents, N contents in physiological maturity period, straw yields, grain N yields, plant total N yields, N translocation efficiencies, agronomic efficiencies, physiological efficiency, water use efficiencies grain and water use efficiencies for biomass increased by 25.0, 35.7, 0.6, 47.9, 37.7, 25.3, 51.5, 26.6, 51.3 and 5.8% with binary (73 + 82) bacteria inoculation, respectively. Also, N uptake efficiencies of as much as 69.9% and N use efficiencies of as much as 70.7% of chemical fertilizers were achieved with 73 + 82 bacteria treatments. Similar to present findings, bio-fertilizers (NB1 and PB3) showed comparable effects on maize biomass when compared to full chemical fertilization (Lin et al. 2019; Tang et al. 2020); the use of bio-fertilizers was beneficial in improving the growth and productivity of wheat (Singh and Prasad 2011) and barley (Azadikhah et al. 2019; Liu et al. 2022). Almost 50% of the applied N is lost through different ways and 90% of P may not be absorbed by the plants. Nitrogen efficiency rates of cereals were between 29 and 42% (Raun and Johnson 1999). Great emphasis has been given to efficient use of N fertilizer in crop production owing to rising costs of N fertilizers and increasing concern on nitrate pollution in ground and surface water resources. Nitrogen use efficiency in barley can be considered from three inter-related points of view: agronomy, environment and economics. Gang et al. (2021) reported that barley seed inoculated with bacteria exhibited higher N use efficiency as compared to the control plants. The use of plant growth promoting microorganisms could be a feasible solution to improve nitrogen use efficiency. Since these bacteria strains were able to increase the growth and productivity of inoculated barley as compared to non-inoculated plants, they could be potentially used as microbial inoculants (Ibanez et al. 2021).

Dual N-fixating 73 + 82 bacteria combination was followed by triple combination and grain N, straw yield, grain N yield, total N yields, N uptake efficiency, N translocation efficiency, physiological efficiency and water use efficiency for biomass in triple combination were not significantly different from dual bacteria treatment. As compared to the control treatment, grain N contents, N contents in physiological maturity period, straw yields, grain N yields, plant total N yields, N translocation efficiencies, agronomic efficiencies, physiological efficiency, water use efficiencies for grain and water use efficiencies for biomass increased by 29.8, 21.0, 10.9, 50.4, 37.4, 33.8, 35.6, 14.7, 35.7 and 10.6%, respectively, with triple (73 + 82 + M3) bacteria inoculation. In a previous study, the dry matter production of rice and barley increased significantly by 10–20% with the inoculation with the mixture as compared to non-inoculated control (Jamily et al. 2019). It was stated that inoculation with rhizospheric-endophytic mixture strains may be a promising option to improve cereals quality (Emami et al. 2018). Also, N uptake efficiencies of as much as 69.3% and N use efficiencies of as much as 48.9% of chemical fertilizers were achieved with 73 + 82 + M3 bacteria treatments. On the other hand, it was stated by other researchers that the application of bio fertilizers in barley increased the water use efficiency (Singh et al. 2012). In a previous study with wheat, triple bacteria application had similar results with N + P application in terms of both yield and productivity parameters (Bulut 2013a, b). Similar to present

results, bacterial treatments generally showed comparable or higher overall N and P use efficiencies than full chemical fertilization (Tang et al. 2020). These findings suggest that at least half the amount of N and P fertilizers could be reduced through the use of combined fertilization together with beneficial bacteria. Other researchers declared that treatments [N application + phosphorus solubilizing bacteria (PSB) + plant growth promoting rhizobacteria (PGPR)] had no or minimum effects on agro-morphological parameters, but had more prominent effects on soil biological properties (Namli et al. 2017).

Conclusion

Chemical fertilizers cause harm to the environment as well as their positive effect on crop yields. Therefore, alternative nutrition sources are looked for and biological alternatives are promising for out-yielding. Efficiency parameters of dual (73 + 82) and triple (73 + 82 + M3) bacteria inoculations treatments yielded closer outcomes to chemical fertilizers (N + P recommended dose). These findings suggest that at least half the amount of N and P fertilizers could be reduced through the use of combined fertilization together with beneficial bacteria. Finally, the combination of bacteria inoculation (binary: 73 + 82 and triple: 73 + 82 + M3) could become one of the most charming option concerning soil enhancement and minimizing chemical fertilization in organic barley cultivation.

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Author Contribution Özcan Çağlar: Project Administration and Supervision, Conceptualization, Methodology, Resources, Review. Sancar Bulut: Software, Formal analysis, Data Curation, Writing—original draft and Visualization & Editing.

Declarations

Conflict of interest Ö. Çağlar and S. Bulut declare that they have no competing interests.

Ethical standards This paper contains findings of an original research. Applicable national and/or international guidelines for animal ethics were followed and no animal was harmed during the study. Ethical standards were adhered in this study and in production of this manuscript.

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