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Evaluation of efficiency parameters of phosphorous-solubilizing and N-fixing bacteria inoculations in wheat (*Triticum aestivum* L.)

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Abstract: Chemical fertilizers play a significant role in increasing plant production; however, they may have a negative impact on soil fertility and cause environmental degradation in cases of excessive use. In addition, the highest levels in chemical fertilizer use have already been reached, and further yield increases do not seem possible. Due to their negative impact on soils and the environment, alternatives to chemical fertilizers are needed. Today, biological alternatives are yielding promising outcomes. Biological nitrogen fixation may constitute a significant alternative in organic farming. Therefore, in the present study, the effects of single, dual, and triple combinations of phosphorus-solubilizing [*Bacillus megaterium* var. *phosphaticum* (M-13)] and nitrogen-fixing bacteria [*Stenotrophomonas maltophilia* (82) and *Ralstonia pickettii* (73)] treatments were compared with chemical fertilizer and control treatments with regard to wheat efficiency parameters. The efficiency parameters of the triple bacteria inoculation (73 + 82 + M-13) treatment were close to those of chemical fertilizers: up to 94.3% of the nitrogen uptake efficiency, 94.1% of the nitrogen translocation efficiency, 85.9% of the nitrogen use efficiency, 91.9% of the agronomic efficiency, and 91.7% of the water use efficiency of chemical fertilizers were reached. In terms of efficiency, the triple combination (73 + 82) was followed by the nitrogen-fixing dual bacteria treatment. Therefore, the triple bacteria combination may be recommended as an alternative fertilization method in organic wheat farming.

Key words: Bacteria inoculation, efficiency parameters, wheat, organic farming

1. Introduction

Considerable developments in environmental protection and social health issues have been observed worldwide. As with global warming or climate change, environmental pollution is a transboundary problem. Considering the limited resources of the earth, sustainability and environmental protection efforts have gained great significance. In addition, rapid increases in population speed up environmental degradation and reduce living standards. A 'green revolution' was the main goal of agricultural policies designed to meet the food demands of rapidly increasing populations, especially during the 1960s and 1970s. The main theme of such a revolution is to increase yields per unit area through high-yield cultivars and intensive use of fertilizers and plant protection chemicals. In developed countries, the promotion of chemical fertilizer and pesticide use has become national policy. Because intensive chemical input has led to some negative impacts, environmentally friendly production systems have been explored as alternatives to traditional high-input systems (Kantar, 1997; Bulut, 2009). This search for alternatives has produced the terms 'organic' and 'sustainable agriculture'. Since organic agriculture systems are not sufficient to meet world needs, sustainable agriculture has instead gained great attention and support. Sustainable agriculture coordinates the capacities of natural resources to meet future demand by reducing the degradation of natural resources and promoting biological processes and diversity instead of nonagricultural inputs (Haktanır et al., 1995). Bioorganic systems play a significant role in the development and implementation of sustainable agricultural techniques. Cereals cover the greatest area worldwide, and an efficient microorganism–host combination is highly effective for reducing the degradation of natural resources through nitrogen fixation. This combination also brings significant economic advantages.

Free-living bacteria depend on soil organic matter as a food source, and their activities increase under the proper conditions. These bacteria promote plant growth through their nitrogen-fixing and phosphorus-resolving abilities in combination with their natural growth hormones. The impact is higher in greenhouses with proper environmental and irrigation conditions. Therefore, biological fertilization

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can be an alternative to mineral fertilization (Çakmakçı et al., 1999). Agriculture-borne plant residues, green fertilizers, manure, and municipal and industrial waste materials may also be used as sources of organic fertilizer. Hay, manure, molasses, and starch-like materials increase the number of phosphate-solubilizing bacteria and P amounts available in the rhizosphere. The application of organic material to the soil increases microbial populations, microbial activity, and enzyme activity (Martyniuk and Wagner, 1978). Microorganisms constitute about 1%–3% of total organic matter and function as catalyzers in the decomposition process (McGill et al., 1986). Correlations of phosphate activity with organic matter and organic P concentrations in different cultural systems were reported by Lima et al. (1996). It was also reported that organic matter and chemical fertilizer applications increased soil microbial populations and microbial enzyme activity, and organic fertilizers had a greater impact on microbial populations than chemical fertilizers (Goyal et al., 1992).

Nitrogen has a vital importance for plant growth, but it is also one of the main factors limiting agricultural productivity in several countries. Nitrogen is taken in by plants basically in the form of nitrate and ammonium, and it constitutes the basic building block of proteins. However, the energy sources needed to produce nitrogenous fertilizers are being rapidly depleted, both in Turkey and in the rest of the world. Therefore, microorganisms that are able to fix atmospheric nitrogen into soil are used more commonly to meet the nitrogen demands of plants and reduce or totally eliminate nitrogen applications to the soil. The second most important nutrient after nitrogen in agricultural production is phosphorus. It is the most immobile nutrient in soil; therefore, its intake by plants is both small and slow (Mengel et al., 2001). It also has a synergic effect on nitrogen intake. Phosphorus is present in soils, especially as low water-soluble phosphates (mainly Ca and Fe phosphates). Inoculation with *Bacillus megaterium* var. *phosphaticum*, a phosphorus-dissolvent bacteria, increases the available phosphorus levels of soils (Saber, 1997; Schilling et al., 1998).

Artificial fertilizer use in plant production is not always reliable or economical in arid and semiarid regions with low precipitation. In addition, yield is lower in these regions due to ecological conditions, and the economic power of farmers is low and agricultural practices are not sufficient. Each of these factors also limits the use of fertilizer in agricultural activities. Nitrogen fixated into the soil through biological processes has higher impact rates than nitrogen applied through commercial fertilizers. This provides some advantages in arid and semiarid regions (Çelik and Uzun, 1992).

Proper plant nutrition and fertilizer use efficiency in modern agriculture mostly depend on the proper establishment of plant nutrition strategies. Several factors are effective in proper plant nutrition strategies. For instance, plant nutrition techniques are selected so that nutrient losses from soils through leaching, denitrification, volatilization, and runoff are minimized and fertilizer use efficiency is increased. Unconscious fertilizer use may cause nitrogen leaching or volatilization, and phosphorus- and potassium-like nutrients may become useless forms of these nutrients (Barlog and Grzebisz, 2004). Almost 50% of the nitrogen applied to soil is lost through various means, and 90% of phosphorus may not be taken up by plants. The nitrogen efficiency rate was reported to be between 29% and 42% for cereals (Raun and Johnson, 1999).

The present study was conducted to reduce the dependency on chemical fertilizers in spring wheat culture in Erzurum, Turkey. Effects of phosphorus-solubilizing (*Bacillus megaterium* var. *phosphaticum*) and nitrogen-fixing asymbiotic (*Stenotrophomonas maltophilia* and *Ralstonia pickettii*) bacteria strains on nitrogen intake and use efficiency parameters were investigated by field experiments. Comparisons were also made between the effects of single and combined bacteria treatments.

2. Materials and methods

2.1. Location, design, and treatments

Experiments were carried out in the experimental fields of Agricultural Research and Extension Center of the Atatürk University Agricultural Faculty in 2004 and 2005. The Kırik wheat cultivar was used as seed material. Phosphorus-solubilizing *Bacillus megaterium* var. *phosphaticum* (M-13) and high nitrogen-fixing *Stenotrophomonas maltophilia* (82) and *Ralstonia pickettii* (73) bacteria strains supplied by the Plant Protection Department of the Atatürk University Agricultural Faculty were used for inoculations. These high nitrogen-fixing strains (73 and 82) were isolated in a previous study from the roots of cereal crops grown in Erzurum and the Pasinler Plains. The effects of single and combined bacteria treatments on wheat were compared with control (uninoculated and unfertilized) and chemical fertilizer treatments (Table 1). The recommended nitrogen dose of 80 kg ha^{-1} was supplied as ammonium sulfate (21% N) and a phosphate dose of 50 kg ha^{-1} was supplied as triple superphosphate (42% P_2O_5) (Köycü, 1974; Akkaya, 1993). The entire P dose was applied at sowing, and nitrogen was applied in 2 equal doses during the soil preparation at sowing and at the beginning of stem elongation.

Experiments were carried out in a randomized block design with 3 replications. A total of 11 treatments were randomly distributed among plots in each block. There were a total of 33 plots of 6.0 × 1.2 m and 6 plant rows 20 cm apart. Therefore, the total plot area was $1.2 \times 6 = 7.2$ m^2. A distance of 2 m was left between blocks, and 0.4 m was left between the plots.

Table 1. Field experiment treatments.

Abbreviations	Treatments
Control	Uninoculated and unfertilized
M-13	*Bacillus megaterium* var. *phosphaticum* (inoculation of phosphorus-solubilizing strain M-13)
73	*Ralstonia pickettii* (inoculation of N-fixing asymbiotic strain 73)
82	*Stenotrophomonas maltophilia* (inoculation of N-fixing asymbiotic strain 82)
M-13 + 73	*B. megaterium* + *R. pickettii*
M-13 + 82	*B. megaterium* + *S. maltophilia*
73 + 82	*R. pickettii* + *S. maltophilia*
M-13 + 73 + 82	*B. megaterium* + *R. pickettii* + *S. maltophilia*
N	80 kg ha^{-1} nitrogen as ammonium sulfate (21% N) applied
P	50 kg ha^{-1} phosphorus as triple superphosphate (45% P_2O_5) applied
N + P	Combined N and P application

2.2. Seed inoculation

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

2.3. Crop management and measurements

Seed beds were prepared in the spring and inoculated with bacteria. Previously cultured bacteria were propagated into seeds through sugared water and inoculated/uninoculated seeds were hand-sown and planted at 525 seeds m^{-2}. Gloves were replaced for each strain to prevent contamination. For the chemical fertilizer treatments, all of the phosphorus and half of the nitrogen were applied with sowing; the remaining nitrogen was applied at the bolting period. Fertilizers were applied by hand and spread between plant rows. Hand weeding was performed in all plots during the tillering period. The border strip irrigation method was applied 3 times: at bolting, at the beginning of heading, and in the middle of the milky ripe period, in order to saturate the soil and, consequently, prevent bacterial growth.

Following full ripening, plants were harvested manually with hooks from 4 m^2 of each plot. A row from each side and 50-cm strips from the beginning and end of the plots were eliminated as side effects. Harvested plants were sheaved and left on the ground to dry for 3 days. The dried plants were then threshed by plot thresher.

2.4. Climate and soil characteristics

The climate parameters for the experimental years are presented in Table 2. The year 2005 seemed more suitable for wheat growth with regard to total precipitation and average temperature.

Table 2. 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 supplied by the Erzurum Regional Directorate of Meteorology. LYM, 1990–2005: long years mean.

The physical and chemical characteristics of the experimental soils are presented in Table 3. The organic matter content of the experimental soils was 1.5%–1.6%, lime content 2.7%–3.1%, and pH 7.6. Available phosphorus and potassium levels in 2004 and 2005 were 22.7–34.3 and 215.8–206.3 kg ha^{-1}, respectively (Toptaş, 1987).

2.5. Measurements and statistical analysis

The following parameters were determined: grain N (%), physiological maturity N (%), straw yield, grain N yield, total N yield, N uptake efficiency (NUpE), N translocation efficiency (NTE), N use efficiency (NUsE), agronomic efficiency (AE), physiological efficiency (PE), water use efficiency for grain (WUE_g), and water use efficiency for biomass yield (WUE_b). Percentage N was determined using the Kjeldahl method (American Association of Cereal Chemists, 1983). Data were subjected to analysis of variance using the MSTAT-C software package. Duncan's multiple range test was performed to determine the differences among the treatments (P = 0.01).

3. Results

Differences between years and between treatments were significant with regard to grain N content. While grain N content in 2004 was 2.99%, the value was 2.23% in 2005. Grain nitrogen content was higher in 2005 due to the higher precipitation that year. Grain N contents of the control, M-13, 73, 82, 73 + M-13, 82 + M-13, 73 + 82, 73 + 82 + M-13, N, P, and N + P treatments were 2.35%, 2.05%, 2.68%, 2.55%, 2.37%, 2.46%, 2.72%, 2.79%, 2.94%, 2.75%, and 3.07%, respectively. Although the highest values were observed in N + P, single nitrogen, and 73 + 82 + M-13 bacteria treatments, the differences among these treatments were not significant. The lowest values were seen in control treatments without any fertilizer or bacteria application (Table 4). Compared to the control treatment, grain N content increased by 18.7% under the 73 + 82 + M-13 bacteria treatments.

Differences between years and between treatments were significant with regard to nitrogen content in the physiological maturity period; the value was 0.663% in 2004 and 0.531% in 2005. The value was lower in 2005 again due to the higher precipitation levels that year. The highest values were observed in N + P treatments. The lowest values were seen in the single M-13 bacteria and control treatments without any fertilizer or bacteria application. With the 73 + 82 + M-13 bacteria treatment, N content in the physiological maturity period increased by 18.6% compared to the control. Differences between years and treatments were significant with regard to straw yield; average straw yield was 6111.0 kg ha^{-1}. A higher yield was observed in 2005 due to the higher precipitation that year. While the highest values were observed in N + P, single N, and triple bacteria treatments, the lowest value was seen in the control treatment (Table 4). Compared to the control, 55.5% higher straw yields were observed in the triple bacteria treatments (73 + 82 + M-13).

Differences between years and between treatments were significant with regard to grain N yield. While grain N yield in 2004 was 62.4 kg ha^{-1}, the value was 49.3 kg ha^{-1} in 2005. While the highest values were observed in N + P and single N treatments, the lowest value was seen in the control treatment (Table 4). Compared to the control treatment, 73.1%, 78.1%, and 78.4% higher grain N yields were observed in 82, 73 + 82, and 73 + 82 + M-13 bacteria treatments, respectively. Differences between years and between treatments were significant with regard to plant total nitrogen yield. While the highest values were observed in N + P and single N treatments, the lowest value was seen in the control treatment (Table 4).

Differences between years were found to be insignificant with regard to NUpE. However, differences between treatments were significant. While the highest values were observed in N + P, N, and triple bacteria treatments, respectively, the lowest value was seen in the single M-13 bacteria treatment (Table 4). Nitrogen uptake efficiencies of up to 94.3% of that of chemical fertilizer treatments were achieved in triple bacteria treatments. Differences between years were not significant with regard to NTE. In terms of this value, the average of the 2 years was 60.3%. However, differences between treatments were found to be significant. While the highest value was observed in the N + P treatment, the lowest value was seen in the control treatment (Table 5). Compared to the control treatment, 23.6% and 29.4% higher nitrogen translocation efficiencies were observed in 73 + 82 and 73 + 82 + M-13 bacteria treatments, respectively.

Table 3. 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	27.0	7.6	1.5	3.1	22.7	2158
2005	Clay-loam	30.6	37.3	29.2	7.6	1.6	2.7	34.3	2063

*Soil analyses performed in the laboratories of the Soil Science Department of the Atatürk University Agricultural Faculty.

Table 4. Effects of experimental variables on grain N content, physiological maturity N content, straw yield, grain N yield, total N yield, and N uptake efficiency.[1]

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	2.99 a	0.663 a	5937.8 b	62.4 a	102.0 a	41.65
2005	2.23 b	0.531 b	6283.3 a	49.3 b	82.8 b	40.41
Mean	2.61	0.597	6111.0	55.9	92.4	41.03
Treatment (T)						
Control	2.35 e	0.533 d	4625.0 f	33.8 f	62.6 h	-
M-13	2.05 f	0.527 d	5900.0 cde	40.9 e	79.5 g	21.16 g
73	2.68 cd	0.625 ab	5807.8 de	56.4 cd	91.7 def	36.48 def
82	2.55 de	0.620 ab	5392.0 ef	58.5 c	93.6 cde	38.81 cde
73 + M-13	2.37 e	0.545 cd	5266.7 ef	56.1 cd	84.8 fg	27.86 fg
82 + M-13	2.46 e	0.587 bc	5116.7 ef	53.2 d	86.2 efg	29.60 efg
73 + 82	2.72 cd	0.628 ab	6366.7 bcd	60.2 c	100.8 abc	47.77 abc
73 + 82 + M-13	2.79 bc	0.632 ab	7183.3 ab	60.3 c	105.0 ab	53.05 ab
N	2.94 ab	0.637 ab	7183.3 ab	66.3 b	106.1 a	54.42 ab
P	2.75 bcd	0.585 bc	6767.8 abc	57.7 cd	98.5 bcd	44.94 bcd
N + P	3.07 a	0.650 a	7607.8 a	71.1 a	107.5 a	56.25 a
LSD	0.19	0.05	870.02	4.7	7.0	9.04
F values						
Y	626.81***	2032.59***	63.2*	3224.0***	2999.6***	0.68
T	33.88***	84.36***	184.6***	748.5***	542.5***	26.78***
Y × T	2.58	0.59	59.4***	57.8***	51.4***	5.42***
CV (%)	4.70	1.99	9.14	5.33	4.88	14.08

[1]: Means with the same letter within a 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.

The effects of year and treatment on NUsE were significant, and higher values were observed in 2005 due to higher precipitation levels. While the highest efficiencies were observed in N + P and triple bacteria treatments, the lowest value was seen in the single phosphate-solubilizing M-13 bacteria treatment (Table 5). The effects of years and treatments on AE were both significant. While the highest values were observed in N + P and N treatments, the lowest values were seen in the control and the phosphate-solubilizing M-13 bacteria treatments (Table 5).The effects of year and treatment on PE were significant. The highest value was obtained from the M-13 bacteria treatment and the lowest from N + P fertilizer treatment. The effects of year and treatment on WUE_g and WUE_b were significant. While the highest value was observed in N + P fertilizer treatments, the lowest value was seen in the control treatment for both parameters (Table 5).

4. Discussion

In the present research, variance analysis revealed significant differences between years (except for NUpE and NTE) and treatments with regard to almost all parameters. More favorable climate conditions increased straw yield, WUE_g, and WUE_b but decreased grain N content (%),

Table 5. Effects of experimental variables on N translocation efficiency, N use efficiency, agronomic efficiency, physiological efficiency, water use efficiency for grain, and biomass.[1]

Variable	N translocation efficiency (NTE, %)	N use efficiency (NUsE, kg yield kg^{-1} N)	Agronomic efficiency (AE)	Physiological efficiency (PE)	Water use efficiency for grain (WUE$_g$)	Water use efficiency for biomass (WUE$_b$)
Years (Y)						
2004	60.9	8.2 b	26.0 b	20.5 b	12.5 a	48.3 a
2005	59.7	11.2 a	27.5 a	26.9 a	10.7 b	41.1 b
Mean	60.3	9.7	26.8	23.7	11.6	44.7
Treatment (T)						
Control	51.7 d	-	18.0 f	23.4 cdef	7.8 e	33.0 e
M-13	53.9 cd	7.3 e	25.3 e	27.1 a	10.9 d	40.4 d
73	57.6 c	9.9 bc	25.9 de	24.4 bcd	12.0 bc	43.7 cd
82	56.8 c	9.8 bc	27.8 bc	23.6 cde	11.5 cd	43.0 cd
73 + M-13	55.9 cd	9.3 bcd	27.8 bc	25.9 ab	11.9 bc	40.7 d
82 + M-13	56.8 c	8.7 cde	27.3 bcd	25.1 abc	12.0 bc	40.7 d
73 + 82	63.9 b	10.2 bc	28.1 bc	22.3 def	12.2 bc	46.5 bc
73 + 82 + M-13	66.9 b	11.0 b	28.2 bc	21.6 ef	12.2 bc	50.4 ab
N	65.8 b	10.2 bc	28.9 b	21.3 ef	12.6 b	52.3 a
P	62.7 b	7.9 de	26.6 cde	24.8 abc	11.1 d	47.4 bc
N + P	71.1 a	12.8 a	30.7 a	21.2 f	13.3 a	53.1 a
LSD	4.07	1.58	1.55	2.13	0.67	4.68
F values						
Y	3.76	140.68***	40.28***	367.97***	302.75***	94.86***
T	32.87***	14.28***	65.21***	12.26***	64.59***	24.23***
Y × T	3.85***	12.32***	12.59***	3.19**	12.99***	5.74***
CV (%)	4.34	10.36	3.71	5.76	3.73	6.73

[1]: Means with the same letter within a variable are not significantly different (Duncan's multiple range test, $P < 0.05$).
** and ***: Significant at 0.01 and 0.001 levels, respectively.

physiological maturity N content (%), grain N yield, total N yield, NUsE, AE, and PE. Examined as an average of years, all parameters were significantly influenced by the treatments (Tables 4 and 5). Year × treatment interactions were significant for most of the parameters, mainly due to the varying effects of bacteria in 2004 and 2005.

Compared to the control, significant increases were observed over all parameters with bacteria inoculations (except for grain N and physiological maturity N contents with phosphorus-solubilizing M-13 bacteria inoculation). However, dual and triple bacteria combinations yielded better outcomes than single phosphorus-solubilizing or single nitrogen-fixing bacteria treatments. Moreover, the single phosphorus-solubilizing bacteria inoculation treatment (M-13) yielded lower grain and physiological maturity N contents than control; however, the differences were not significant. Research revealed enriched plant yield, and N and P intake with combined phosphorus-solubilizing bacteria and *Azotobacter* strains (Kundu and Gaur, 1984; Monib et al., 1984). Previous studies also reported that combined culture inoculants significantly increased grain and dry matter yields as compared with single inoculations of individual organisms in sorghum (Alagawadi and Gaur, 1992), sugar beet and barley (Çakmakçi et al., 1999), and tea (Çakmakçı et al., 2012). Others, however, suggested that mixed inoculations had no comparative advantage over single cultures in wheat (Han and New, 1998) and other crops (Chiarini et al., 1998). P-solubilizing bacteria in combination with N_2-fixing organisms were expected to improve the P nutrition of plants and, therefore, stimulate plant growth (Whitelaw et al., 1997). Enhancement of cereal crop yields by inoculation with nitrogen-fixing bacteria was observed in many experiments (Çakmakçi et al., 2001; Ozturk et al., 2003; Salantur et al., 2006). Inoculation of wheat with plant-growth–promoting bacteria increased straw yield (Ozturk et al., 2003), N uptake and N yield (Bhattarai and Hess, 1993), N concentration, and antioxidant enzyme activities (Çakmakçı et al., 2007b) in wheat.

In both years, N + P applications yielded the highest grain N content, physiological maturity N content, straw yield, grain N yield, total N yield, NUpE, NTE, NUsE, AE, WUE_g, and WUE_b. Single N and the mixture of 3 bacteria inoculation treatments yielded lower values, and the control treatment yielded the lowest values with regard to these parameters. Among bacteria treatments, the triple bacteria combination yielded the best results in all parameters, except for PE. The outcomes for bacteria treatments were closer to the values of chemical fertilizer treatments. The differences between the physiological maturity, N content, straw yield, total N yield, NUpE, WUE_b, and PE of bacteria treatments and N + P treatment were not significant. Moreover, the triple bacteria treatment yielded better results across all parameters than the single P treatment, with the exception of PE. Compared to the control, grain N contents, N contents in the physiological maturity period, straw yields, grain N yields, plant total N yields, nitrogen translocation efficiencies, agronomic efficiencies, grain water use efficiencies, and plant water use efficiencies for biomass increased by 18.7%, 18.6%, 55.5%, 78.4%, 67.7%, 29.4%, 56.7%, 56.4%, and 52.7%, respectively, with triple (73 + 82 + M-13) bacteria inoculation. In addition, nitrogen intake efficiencies of up to 94.3% and nitrogen use efficiencies of up to 85.9% of chemical fertilizers were achieved with 73 + 82 + M-13 bacteria treatments. These values were still behind those obtained with N + P fertilization. Similarly, Dalla Santa et al. (2004) reported insignificant differences in wheat grain total N contents between bacteria inoculation and chemical fertilizers. Baldani et al. (1983) reported 30%–51% increases in plant nitrogen content at flowering compared to the control by inoculation with *Azospirillum* strains. Distinctive superiority of high grain N content treatments over other treatments was reported by Çağlar (1995), Salantur (2003), and Stanojković et al. (2012), and a 19% increase in biomass and a 25% increase in nitrogen yield compared to the control were also reported with bacteria inoculation in wheat (Salantur et al., 2006). A 32.8% increase in straw yield with bacteria inoculation was reported by Darmwal and Gaur (1988), and a 16.0% increase in N yield was reported with dual bacteria inoculation by Rai and Gaur (1988). Increases in total N yield as a result of bacteria inoculations may be explained through the mechanisms of N fixation, P solubilization, and the siderophore and indole acetic acid production abilities of bacteria strains alone and/or in combination, as reported in other studies (O'Hara et al., 1981; Haahtela et al., 1988; Murty and Ladha, 1988; Bhattarai and Hess, 1993). In addition to nitrogen fixation, some bacteria have hormonal impacts on plant growth and are able to release auxin and gibberellin-like compounds into culture medium (Gutierrez Manero et al., 2001). These compounds stimulate root development and increase plant water and nutrient intake by increasing shoot and leaf numbers in the early stages, and, consequently, they positively affect plant growth and yield (Germida and Walley, 1996; Ryder et al., 1999). Similarly, it was reported that bacteria inoculation increased the number of capillary roots and root weight (Haahtela et al., 1988; Gouzou et al., 1993), enhanced soil aggregate stability (Bethlenfalvay et al., 1997), increased root nitrogen uptake (Saric et al., 1987; Rai and Gaur, 1988; Saubidet et al., 2002; Çakmakçı et al., 2006, 2007a), and increased nitrogen yields (Bhattarai and Hess, 1993). Nitrogen use efficiency is defined as the percentage of regained nitrogen at harvest of the amount applied through fertilization (Pollmer et al., 1979). Improved NUsE may provide increases in kernel yield but decreases in the protein ratio (Bulut et al., 2013). The efficient use of N fertilizer is becoming increasingly important in crop production due to the rising costs of N fertilizers and growing concern about the nitrate pollution of underground and surface water resources. Nitrogen use efficiency in wheat can be considered from 3 interrelated points of view: agronomy (in terms of grain yield produced per unit of N applied), the environment (possible contamination of ground water, eutrophication of surface waters), and economics (maximization of farmer income) (Raun and Johnson, 1999; Foulkes et al., 2009; Bayeh, 2010). Maximizing NUsE will help to produce high yields with low N inputs (Limon-Ortega et al., 2000; Lewandowski and Schmidt, 2006; Malézieux et al., 2009; Hirel et al., 2011; Bulut, 2013) without creating watercourse pollution (Semenov et al., 2007; Huggins et al., 2010). Therefore, there is an increasing emphasis worldwide on developing wheat N management strategies (Shanahan et al., 2008; Foulkes et al., 2009) for high NUsE. Grain yield was positively correlated with NUsE, N content, and the N translocation ratio, whereas the N translocation ratio was correlated with grain protein concentration. Rodrigues et al. (2000) reported that bacteria inoculation did not affect assimilate translocation; however, translocation increased with increasing nitrogen. This is in concordance with the findings of the current study, which observed the highest NTE with N + P treatment and the lowest value in the control treatment (no bacteria inoculation or fertilizer treatment). Contrary to studies reporting increased nitrogen uptake and use, Şahin et al. (2010) reported the negative impacts of inoculation on NUsE.

The triple combination was followed by the dual nitrogen-fixing 73 + 82 bacteria combination; however, N contents in the physiological maturity period, grain N yields, plant total N yields, NUpE, NTE, AE, and WUE_g in the dual bacteria treatment were not significantly different from the triple combination. Compared to the control treatment, grain N contents, N contents in the physiological maturity period, straw yields, grain N yields, plant total N yields, NTE, AE, WUE_g, and WUE_b increased under dual (73 + 82) bacteria inoculation by 15.7%, 17.8%, 37.8%, 78.1%, 61.0%, 23.6%, 56.1%, 56.4%, and 40.9%, respectively. In addition, nitrogen intake efficiencies of up to 84.9% and nitrogen use efficiencies of up to 79.7% of those of chemical fertilizers were achieved with the 73 + 82 bacteria treatment. However, dual combinations of phosphorus-solubilizing and nitrogen-fixing bacteria (73 + M-13, 82 + M-13) did not yield good outcomes. Such outcomes from dual bacteria treatments were also reported by other researchers (Chiarini et al., 1998; Han and New, 1998). Mixed microbial cultures allow their components to interact with each other synergistically, and the interaction of N_2-fixing bacteria with other bacteria could also inhibit their diazotrophic activity or plant growth (Rojas et al., 2001; Oliveira et al., 2002; Şahin et al., 2004).

In conclusion, although chemical fertilizers play a significant role in increasing plant production, they may have negative impacts on soil fertility and cause environmental degradation in cases of excessive use. The highest levels for chemical fertilizer use have already been reached; further yield increases are not possible. Therefore, alternatives to chemical fertilizers are needed, and biological alternatives are yielding promising outcomes. Biological nitrogen fixation may constitute a significant alternative in organic farming. In the present study, the efficiency parameters of a triple bacteria inoculation treatment were closer to those of chemical fertilizers, and as much as 95.1% of the NUpE, 94.1% of the NTE, 91.9% of the AE, and 91.7% of the water use efficiency of chemical fertilizers were reached with bacteria treatments. The triple combination was followed by the nitrogen-fixing dual bacteria treatment (73 + 82) in terms of these parameters. Therefore, the triple bacteria combination (73 + 82 + M-13), which provided efficiencies of up to 90% of N + P fertilization in organic farming, may be recommended as an alternative fertilization method for organic farming.

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