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Evaluation of yield and quality parameters of phosphorous-solubilizing and N-fixing bacteria inoculated in wheat (*Triticum aestivum* L.)

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Abstract: Considering current agricultural practices and certain indicators, it is impossible to comment on sustainability of these practices. Chemical fertilizers are among such unsustainable practices. Because of increasing mineral fertilizer costs and negative environmental impacts of these fertilizers, the interest in biological nitrogen fixation is increasing within the scope of sustainable agriculture (without significant yield losses). Therefore, effects of phosphorus-solubilizing (*Bacillus megatherium* var. *phosphaticum* [M-13]) and nitrogen-fixing (*Stenotrophomonas maltophilia* [82] and *Ralstonia pickettii* [73]) bacteria and chemical fertilizer treatments on wheat yield and quality parameters were compared with control treatment in present study. Significant differences were observed among treatments with regard to entire parameters. Although the best results were observed in chemical fertilizer treatments, single, dual, and triple bacteria combinations yielded significant increases in grain filling period, number of spikes per square meter, number of kernels per spike, 1000-kernel weight, biological yield, grain yield, grain protein ratio, plant protein ratios in flowering, and physiological maturity periods. Based on these findings and considering the M-13 + 73 + 82 triple bacteria combination with regard to grain yield and protein ratios, it may be concluded that such treatments may reduce the amount of fertilizer to be used by 20%. Among the dual bacteria treatments, 73 + 82 and 82 + M-13 were found to be advantageous with regard to grain filling period, 73 + M-13 with regard to 1000-kernel weight and grain protein ratio, and M-13 + 73 and M-13 + 82 with regard to plant protein ratio in the physiological maturity period.

Key words: Inoculation, rhizobacteria, yield response, wheat

1. Introduction

Cereal crops constitute the largest product group with their cultivation and production levels around the world. Almost half of the world's cultivated land resources are allocated for cereal farming. Wheat was cultivated over 217.2 × 10^6 ha in the world in 2010 and total production was realized as 653.7 × 10^6 t with an average yield of 3009 kg ha^{-1} (http://www.fao.org). Cereal farming is carried out on over 75% of cultivated lands in Turkey, and wheat (68.0%) and barley (22.3%) have the largest share among cereal crops. Current data indicate wheat farming lands of Turkey as 8.1 × 10^6 ha and total production as 21.8 × 10^6 t with an average yield of 2785 kg ha^{-1} (http://www.tuik.gov.tr). Dry farming is practiced in the wheat culture of Turkey. Therefore, the amount and distribution of precipitations are the main factors affecting the yield. Wheat yield of Turkey is usually lower than world averages.

Precipitation regime is the most significant factor effective on production in northeastern Anatolia. Beside the precipitation, poor soils with regard to plant nutrients are also a significant concern for low yield in field farming. In fact, soils of the northeastern and southeastern Anatolia regions, including Erzurum Province, are mostly poor in organic matter and available phosphorus. For years, monocultural practice has caused significant losses in soil fertility and yield of the region. Nitrogen gain through organic material mineralization is a critical issue for regional soils to overcome nitrogen deficiency. Various fertilization implementations (chemical, manure) or bacteria inoculation with proper strains should be carried out to improve the soil fertility and corresponding yield values. After nitrogen, phosphorus is the second most critical plant nutrient in plant production. Phosphorus is the least immobile nutrient in soils. Therefore, intake rates and amounts are low in plants (Mengel et al., 2001). However, phosphorus has a synergic impact in nitrogen intake. Phosphorus presents in soils especially as low water-soluble phosphates (mainly as Ca and Fe phosphates). Inoculation with *Bacillus megatherium* var. *phosphaticum*, like phosphorus dissolvent, plays a significant role in

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increasing available phosphorus levels of soils (Saber, 1997; Schilling et al., 1998; Saharan and Nehra, 2011).

High chemical fertilizer costs and low income levels of regional farmers limit the fertilizer use of farmers. Provincial data indicate that there was no fertilization over almost half of the cereal-cultivated lands of Erzurum, and implementation rates were very low in fertilized sections (average: 33 kg ha^{-1} nitrogen, 20 kg ha–$^{-1}$ phosphorus) (http://www.tuik.gov.tr). Chemical fertilizers are high-cost inputs in developing countries. Turkey pays a large sum of exchange each year for fertilizers. External dependence increases fertilizer prices each year and such an increase will probably continue in upcoming years. Intensive fertilizer use for high yield increases production costs. Precipitation dependency of mineral nitrogenous fertilizers (Gauer et al., 1992), easy leaching of such fertilizers and consequent environmental and water pollution (Daneshmand et al., 2012), and transformation of phosphorus into low water-soluble compounds and unavailable forms for plants (through compounding with Fe and Al in acidic soils and with Ca in alkaline soils) all indicate that productivity of agricultural lands is not totally dependent on chemical fertilizers (Kantar, 1997). Research has shown that due to quick nitrogen loss in many different ways plants only get a small portion of nitrogenous fertilizers applied to soil (Broadbent and Nakashima, 1968; Terman and Brown, 1968). Availability of phosphorus in soil is also very low and plants may uptake only a small portion of this available phosphorus. Phosphorus availability is also significantly affected by soil pH (best intake at pH 6–7.5) and soil type (Saharan and Nehra, 2011).

Environmentally friendly production systems are gaining in popularity to overcome the negative aspects of traditional production techniques with intensive inputs (Kantar, 1997). Organic and sustainable agricultural practices have become more widespread around the world (O'Connell, 1992; Orson, 1996). Bioorganic systems play crucial roles in the development and implementation of sustainable agricultural techniques (Salantur, 2003). Microbial fertilization is the vital implementation in sustainable agricultural systems to reduce environmental pollution and natural resource deterioration. Symbiotic and asymbiotic nitrogen-fixing bacteria, as well as the bacteria that are able to increase phosphorus solubility and intake, are commonly used for microbial fertilization purposes. New thoughts and approaches will be needed in the formation of the future agricultural policies of Turkey. Therefore, support provided to users of commercial chemical fertilizer should also be provided to users of organic fertilizers, which have several positive impacts on soils and surrounding environments.

The present study was conducted to reduce the dependency on chemical fertilizers in spring wheat farming in Erzurum. Effects of phosphorus solubilizing (*Bacillus megatherium* var. *phosphaticum*) and nitrogen fixing asymbiotic (*Stenotrophomonas maltophilia* and *Ralstonia pickettii*) bacteria strains on plant growth, yield, and quality parameters were investigated in field experiments.

2. Materials and methods

2.1. Location, design, and treatments

Experiments were carried out in the experimental fields of the Agricultural Research and Extension Center of Atatürk University Agricultural Faculty during the years 2004 and 2005. Alternative Kırik wheat cultivar was used as the seed material for the experiments. Phosphorus-solubilizing *Bacillus megatherium* var. *phosphaticum* (M-13) and high nitrogen-fixing *Stenotrophomonas maltophilia* (82) and *Ralstonia pickettii* (73) bacteria strains supplied from the Plant Protection Department of Atatürk University's Agricultural Faculty were used for the seed inoculations. The strains with high nitrogen-fixing capacity (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 the control treatment (uninoculated and unfertilized) and the chemical fertilizer treatments (Table 1). A 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). 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 to plots in each block. There were a total of 33 plots with 6.0 × 1.2 m dimensions and 6 plant rows 20 cm apart. Therefore, the plot area was 1.2 × 6 = 7.2 m^2. A 2-m distance was left between the blocks and 0.4 m was left between the plots.

2.2. 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 1 g L^{-1}; yeast extract 2 g L^{-1}; peptone 5 g L^{-1}; 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 treatments, seeds were placed in bacterial suspensions of 10^8 CFU mL^{-1} for 30 min before sowing.

2.3. Crop management and measurements

Seed beds were prepared in spring and inoculated. Previously cultured bacteria were propagated into seeds

Table 1. Field experiment treatments for seed.

Abbreviations	Treatments
Control	Uninoculated and unfertilized
M-13	*Bacillus megatherium* 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. megatherium* + *R. pickettii*
M-13 + 82	*B. megatherium* + *S. maltophilia*
73 + 82	*R. pickettii* + *S. maltophilia*
M-13 + 73 + 82	*B. megatherium* + *R. pickettii* + *S. maltophilia*
N	80 kg ha^{-1} nitrogen as ammonium sulfate (21% N) was applied
P	50 kg ha^{-1} phosphorus as triple super phosphate (45% P_2O_5) was applied
N + P	Combined N and P application

through sugared water and inoculated-uninoculated seeds were hand-sown by planting 525 seeds m^{-2}. Gloves were changed for each strain to prevent contamination. For chemical fertilizer treatments, all of the phosphorus and half of the nitrogen was applied with sowing, and the remaining half of the nitrogen was applied at the beginning of stem elongation. Fertilizers were applied by hand-spreading between plant rows. Hand weeding was performed in all plots during the tillering period. A border strip irrigation method was applied 3 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.

Following the 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 over land to dry for 3 days. The dried plants were then threshed by a plot thresher.

2.4. Climate and soil characteristics

Total precipitation and average temperatures in May, June, July, and August were 121.7, 40.7, 2.4, and 1.3 mm and 9.7, 14.5, 17.9, and 19.6 °C in 2004, and 92.1, 70.0, 20.3, and 24.3 mm and 10.6, 13.9, 20.2, and 20.4 °C in 2005 (http://www.mgm.gov.tr). The year 2005 seemed to be more suitable for wheat growth with regard to total precipitation and average temperatures. Organic matter content of experimental soils was determined as 1.5%–1.6%, lime content as 2.7%–3.1%, and pH as 7.6. Available phosphorus and potassium levels during 2004 and 2005 were respectively found to be 22.7–34.3 and 215.8–206.3 kg ha^{-1}. Soils of the experimental fields were slightly alkaline with a clay-loam texture, low organic material and lime, moderate phosphorus, and high potassium contents.

2.5. Measurements and statistical analysis

The following parameters were determined: grain filling period, spikes per square meter, kernels per spike, plant height, 1000-kernel weight, hectoliter weight, grain yield, biological yield, harvest index, leaf area per plant, grain protein concentration, heading, and physiological maturity period protein concentration. Leaf area was measured at anthesis with an area meter (CID, Inc., model CI-202). Percentage of N was determined by using the Kjeldahl method (American Association of Cereal Chemists, 1983). Data were subject 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

Variance analysis revealed significant differences between years (except hectoliter weight and harvest index) and treatments with regard to all characteristics investigated. More favorable climatic conditions increased grain filling period, spikes per square meter, kernels per spike, plant height, 1000-kernel weight, grain yield, biological yield, and leaf area per plant but decreased grain protein concentration, heading, and physiological maturity period protein concentration. With regard to years, all parameters were significantly influenced by treatments (Tables 2 and 3). Year × treatment interactions were significant for most parameters mainly due to different effects of bacteria during 2004 and 2005.

Table 2. Effects of years and treatments on grain filling period, spikes per square meter, kernels per spike, plant height, 1000-kernel weight, hectoliter weight, and grain yield.

Parameter	Grain filling period (days)	Spikes m^{-2}	Kernels $spike^{-1}$	Plant height (cm)	1000-kernel weight (g)	Hectoliter weight (kg)	Grain yield (kg ha^{-1})
Years (Y)							
2004	29.3 b	487.3 b	12.2 b	72.4 b	37.7 b	77.1	2079.1 b
2005	30.9 a	515.8 a	14.3 a	92.5 a	39.4 a	76.9	2204.1 a
Mean	30.1	501.5	13.3	82.4	38.6	77.0	2141.6
Treatment (T)							
Control	28.8 b	339.2 e	10.5 ef	81.5 b	35.7 d	76.9 ab	1435.8 f
M-13	29.5 b	501.7 c	13.8 bcd	78.6 b	37.9 bc	76.8 b	2022.0 e
73	30.0 b	498.3 c	13.3 cd	82.9 ab	38.1 bc	77.1 ab	2067.7 de
82	29.5 b	520.0 bc	12.1 def	79.3 b	39.6 ab	77.1 ab	2128.8 cde
M-13 + 73	30.0 b	495.8 c	13.0 cde	83.8 ab	40.1 a	75.5 c	2181.2 bcd
M-13 + 82	30.5 ab	500.8 c	11.4 def	78.3 b	39.7 ab	76.8 b	2223.3 bc
73 + 82	30.5 ab	431.7 d	9.9 f	73.5 b	37.5 cd	77.0 ab	2249.2 bc
M-13 + 73 + 82	30.3 ab	520.0 bc	11.8 def	77.9 b	35.9 d	76.9 b	2254.8 bc
N	31.8 a	607.5 a	18.3 a	93.6 a	41.1 a	78.1 a	2313.7 b
P	29.7 b	529.2 bc	15.6 bc	83.1 ab	39.5 ab	77.3 ab	2225.0 bc
N + P	30.3 ab	572.5 ab	16.2 ab	94.3 b	39.2 abc	77.1 ab	2456.8 a
LSD	1.53	61.30	2.44	11.05	1.76	1.07	123.6
F values							
Y	45.66***	8.64**	31.28***	132.87***	37.97***	0.82	409.7***
T	3.69**	18.98***	16.03***	4.91***	13.92***	2.61*	654.1***
Y × T	1.80	2.19	4.82***	1.45	6.49***	3.55**	127.1***
CV (%)	3.27	7.85	11.82	8.61	2.93	1.20	3.71

Means with the same letter are not significantly different by Duncan's multiple range test at $P < 0.05$.
*, **, and ***: significant at the 0.05, 0.01, and 0.001 levels, respectively.

Grain filling periods during 2004 and 2005 were respectively determined to be 29.3 and 30.9 days. The grain filling periods of control, M-13, 73, 82, 73 + M-13, 82 + M-13, 73 + 82, 73 + 82 + M-13, N, P, and N + P treatments were respectively found to be 28.8, 29.5, 30.0, 29.5, 30.0, 30.5, 30.5, 30.3, 31.8, 29.7, and 30.3 days. The differences both between the years and between the treatments were found to be significant with regard to spikes per square meter. The value was higher in 2005. While the highest value was observed in N (607.5) and N + P (572.5) treatments, the lowest value was seen in the control treatment (Table 2). Single and different combinations of bacteria treatments yielded 27.3%–53.3% increase in number of spikes per square meter compared to the control treatment. As the average of years and treatments, number of kernels per spike was determined to be 13.3. Crop years and treatments had significant effects on number of kernels per spike. The value was higher in 2005 due to favorable climate conditions. While the highest values were observed in N (18.3) and N + P (16.2) treatments, the lowest values were seen in the 73 + 82 bacteria treatment and the control treatment (Table 2).

Average plant height was measured as 82.4 cm and effects of cropping years and treatments on plant height were found to be significant. Higher plant heights were observed in 2005 due to higher precipitation rates in that year. The highest value was observed in single N and P treatments, while the lowest value was seen in the 73 + 82 bacteria combination. Average 1000-kernel weight was determined to be 38.6 g and effects of the years and treatments were found to be significant. Again, the value was higher in 2005 because of higher precipitation rates in that year. While the highest values were observed in single N (41.1 g) and 73 + M-13 bacteria treatments, the lowest values were seen in the control treatment (35.7 g) and the 73 + 82 + M-13 (35.9 g) treatment. The average hectoliter weight was found to be 77.0 kg and the differences between cropping years were not found to be significant. While the highest value was observed in the single N treatment, the lowest value was seen in the 73 + M-13 bacteria treatment (Table 2).

Effects of the years and treatments on grain yield were found to be significant and average yield was found to be 2141.6 kg ha^{-1}. The value was higher in 2005 due to the higher precipitation. The highest grain yields were obtained from single N and N + P treatments and the lowest value was observed in the control treatment without any fertilizer and bacteria treatment (Table 2). Effects of the years and treatments on biological yield were also found to be significant and average biological yield was found to be 8252.4 kg ha^{-1}. The biological yield value was again higher in 2005 due to the higher precipitation. As with grain yield, while the highest value was observed in single N and N + P treatments, the lowest value was seen again in the control treatment. Single or combined microorganisms increased the biological yield. Compared to the control treatment, such increases varied between 21.5% and 45.7%. Average harvest index was determined to be 26.3% and cropping years did not have significant effects on harvest index. The highest harvest indexes were observed in the 73 + 82 (30.9%) and 73 + M-13 (29.4%) treatments, while the lowest values were respectively observed in N + P, 73 bacteria, and control treatments (Table 3).

Years and treatments had significant effects on leaf area, and the average leaf area per plant was found to be 27.8 cm^{-2} $plant^{-1}$. Favorable climate conditions in the second year yielded higher areas. The highest value was observed in the N + P treatment and all of the others were placed statistically into the same group. Effects of the years and treatments on grain protein concentration were found to be significant and the average rate was determined as 15%. This rate was higher in 2004 due to less precipitation. While the highest values were observed in N + P (17.65%) and N (16.88%) treatments, the lowest value was seen in the single M-13 (11.79%) bacteria treatment. Significant differences were observed between grain protein concentration of the control treatment and the treatments M-13, 73, 82, and 73 + M-13. Compared to the control treatment, the grain protein ratios in the 73, 82, and M-13 + 73 treatments increased respectively by 19.15%, 15.96%, and 14.1% (Table 3).

Years and treatments had significant effects on the heading period plant protein ratio, and the average concentration was found to be 9.33%. Again, higher values were observed in 2004 due to lower precipitation. The highest value was seen in the single N treatment and the lowest value was observed in the control treatment. Compared to the control treatment, the heading period plant protein ratio increased by 20.6%–32.4% with bacteria treatments. Years and treatments also had significant effects on the physiological maturity period plant protein ratio. The physiological maturity period plant protein concentration was higher in 2004 due to low precipitation that year. While the highest values were observed in N and N + P fertilization treatments and the triple bacteria combination, the lowest values were respectively obtained from the control, M-13, and 82 bacteria treatments (Table 3).

4. Discussion

Chemical fertilizer and bacteria treatments increased grain filling periods. Longer grain filling periods may be related to higher available N contents. N prolongs grain filling periods by retarding aging in green tissues (Frederik and Camberato, 1995). Fertilization increases tillering ability of the plants and decreases tiller deaths, and consequently allows for the formation of higher numbers of spikes (Singh and Prasad, 2011). Although the increase in number of spikes per square meter with single or combined bacteria treatments was lower than in N, P, and N + P treatments, such increases with bacteria treatments were between 27.3% and 53.3% compared to the control treatments. Inoculation increases organic matter content of soils and improves physical, chemical, and biological characteristics of soils, and consequently enriches the availability and intake of plant nutrients (Singh and Prasad, 2011). In a study with single or combined fertilizer and biofertilizer treatments (Bahrani et al., 2010), the highest spike per square meter value was obtained from ammonium nitrate and urea treatments; single and combined *Azotobacter* and *Mycorrhiza* inoculation treatments significantly increased the number of spikes per square meter compared to the control treatment. The highest number of kernels per spike was obtained from N (18.3) and N + P (16.2) treatments and the lowest values were obtained from 73 + 82 bacteria and control treatments (Table 2). Fertilization improves nitrogen nutrition and increases the number of fertile spikelets and flowers, and consequently increases the number of kernels per spike (Singh and

Table 3. Effects of treatments on biomass yield, harvest index, leaf area per plant, grain protein concentration, heading period protein concentration, and physiological maturity protein concentration.

Parameter	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	8016.9 b	26.1	21.6 b	17.18 a	9.52 a	3.86 a
2005	8487.8 a	26.4	33.9 a	12.83 b	9.15 b	3.10 b
Mean	8252.4	26.3	27.8	15.00	9.33	3.48
Treatment (T)						
Control	6060.8 g	23.8 e	22.8 b	13.47 e	7.43 f	3.09 d
M-13	7922.0 ef	25.6 cde	23.8 b	11.79 f	8.98 e	3.09 d
73	8836.0 bcd	23.3 e	28.9 b	16.05 bc	9.84 b	3.63 b
82	7519.7 f	28.4 abc	21.5 b	15.62 cd	9.26 d	3.16 d
M-13 + 73	7447.8 f	29.4 ab	27.9 b	15.37 cd	9.55 c	3.65 b
M-13 + 82	8680.3 cde	27.3 bcd	24.5 b	14.63 de	8.96 e	3.40 c
73 + 82	7365.8 f	30.9 a	21.3 b	14.15 e	9.48 c	3.41 c
M-13 + 73 + 82	8063.2 def	28.1 abc	25.1 b	13.63 e	9.47 c	3.68 ab
N	9640.2 ab	25.5 cde	29.9 b	16.88 ab	10.13 a	3.72 ab
P	9408.3 abc	24.0 de	30.6 b	15.78 bcd	9.46 c	3.66 b
N + P	9831.7 a	22.7 e	49.0 a	17.65 a	10.12 a	3.81 a
LSD	856.3	3.18	14.22	1.11	0.14	0.13
F values						
Y	121.0**	0.47	29.52***	618.79***	281.62***	1388.53***
T	262.0***	10.73***	4.36**	33.54***	404.83***	61.97***
Y × T	72.4***	4.73***	2.04	2.54	0.17	0.84
CV (%)	6.66	7.77	32.89	4.73	0.97	2.36

Means with the same letter are not significantly different by Duncan's multiple range test at $P < 0.05$.
** and ***: significant at the 0.01 and 0.001 levels, respectively.

Prasad, 2011). Diaz-Zorita and Fernandez-Canigia (2008) reported a 6.1% increase in number of kernels per spike with inoculation treatments. Plant height of wheat varies mostly based on plant genetics, fertilization, precipitation, and soil characteristics. The highest plant height in the present study was observed in single N and P treatments. Average nitrogen availability promotes vegetative development and increases plant height. Positive impacts of fertilization sources on 1000-kernel weight are mainly related to increase in the grain filling period and leaf area indexes (Hasanpour et al., 2012). Compared to the control treatments, bacteria treatments significantly increased 1000-kernel weights in previous studies (Singh and Prasad, 2011; Mohammed et al., 2012). Researchers indicated better results of combined phosphate-solubilizing and nitrogen-fixing bacteria treatments than single treatments and reported increased yields in sorghum and barley with combined bacteria treatments. Hectoliter weight is an indicator of flour yield (Bulut et al., 2013) and is also usually used as a quality indicator in wheat standards.

Therefore, it has commercial significance and higher values are desired in wheat cultivars (Sayaslan et al., 2012). Hectoliter weight in the present study was lower in the first year with drier conditions. Woźniak (2013) indicated that wheat hectoliter weight depends primarily on weather conditions.

Turan et al. (2010) reported significant increase in grain yield of wheat with chemical fertilizer and bacteria inoculations. Mohammed et al. (2012) also indicated increasing plant growth and grain yields in wheat with single and combined treatments of *Azospirillium brasilense* and *Bacillus polymyxa*. Although single and combined inoculation treatments significantly increased biological yield in the present study, the differences among treatments were not significant. Compared to the control treatments, bacteria combinations increased the biological yield by 21.5%–45.7%. Similarly, Turan et al. (2010) reported increasing biological yields in wheat with chemical fertilizer and bacteria treatments. Babaogli et al. (2012) reported increasing number of kernels per cob, 100-kernel weight, and biological yield in corn. A study carried out with bacteria treatments revealed significant effects of *Pseudomonas fluorescens* and *Azospirillum lipoferum* on harvest index ($P < 0.01$) (Khorshidi and Ardakani, 2011). Leaf area is a vital and fundamental parameter for light absorption of a plant and has significant impacts on crop yield. The highest leaf area index in the present study was observed in N + P (3.48) and the lowest in the control treatment (2.14). Nitrogenous fertilizer promoted plant growth and increased leaf area index by forming larger and higher number of leaves (Kiani et al., 2005). The dual inoculation treatment was found to be the best treatment in terms of yield, leaf area index, and grain protein content (Hasanpour et al., 2012).

Higher nitrogen content of soil solution and easy intake caused higher crude protein ratios in traditional systems with mineral fertilizations than organic systems. In the present study, fertilization and bacteria treatments (except the M-13 phosphorus-solubilizing bacteria strain) also increased kernel protein content. Rodrigues et al. (2000) reported significant increases in kernel N content with bacteria treatments as compared to the control treatment. Researchers also reported a 13% increase in protein ratios with an *Azotobacter* + *Mycorrhiza* combined treatment (Bahrani et al., 2010). The highest plant protein concentration in the heading period was observed in N treatments, while the lowest value was seen in the control treatment. All microorganism treatments had significant effects on plant protein content in the heading period and a 20% increase was observed with these treatments. Such an increase was mainly due to improved N and P intake by microorganisms (Çakmakçı et al., 2007). While the highest physiological maturity period plant protein ratio was observed in N, N + P, and triple bacteria combination treatments, the lowest protein ratios were seen in the control, M-13, and 82 treatments. Protein ratios in the physiological maturity period of 73, M-13 + 73, M-13 + 82, and M-13 + 73 + 82 bacteria treatments increased significantly compared to the control treatment, and the triple bacteria combination yielded as much physiological maturity period protein contents as the single N treatment.

The role of *B. megaterium* var. *phosphaticum* in mineral phosphate solubility is well known (Lach et al., 1990; Vazquez et al., 2000). Phosphate solubility is related to organic acid and proton release and consequent NH_4 assimilation (Kucey, 1983; Roos and Luckner, 1984; Asea et al., 1988; Abd-Alla, 1994; Illmer et al., 1995; Whitelaw, 2000). Such relationships may be relevant to phosphatase enzyme release. These enzymes mineralize phosphate (Stevenson, 1986). Amalraj et al. (2012) compared the effects of combined and single treatments of *B. megaterium* var. *phosphaticum* and chemical fertilizers and reported better results for combined treatments. Dhale et al. (2011) observed significant differences in tobacco leaf lengths in combined treatments of *Azospirillum* with chemical fertilizers. Similar findings were observed in various other plants (Jha and Mathur, 1993; Hedge et al., 1999; Selvakumari et al., 2000). Researchers indicated that growth-promoting effects of *Bacillus* bacteria may depend on hormone production (Ito, 1993; Hu and Boyer, 1996; Sheng and Huang, 2001). Available P in soil also affects N intake and use (Kim et al., 2003). Therefore, higher P content in soil is resolved by *B. megaterium*, and consequently N intake increases. In the present study, single and combined *B. megaterium*, *S. maltophilia*, and *R. pickettii* bacteria treatments increased grain yields by 40%–57% compared to the control treatment. Similarly, Smith et al. (1962) reported 0%–70% increase in yields with *Bacillus megatherium* var. *phosphaticum* treatments, but also indicated that such increases may change from region to region. Researchers reported yield increase in potato, tomato, apple, citrus, turnip, sugar beet, broad bean, rice, and wheat with *Pseudomonas putida* and *Ps. fluorescens* bacteria inoculations (Suslov, 1982; Kloepper et al., 1988; Lemanceau, 1992; Kloepper, 1994). The amount of N + P applied to soil may be reduced by about 20% with triple bacteria inoculation. Amalraj et al. (2012) reported 25% decrease in chemical fertilizer use by *B. megaterium* var. *phosphaticum* inoculation. The triple bacteria combination may also reduce single nitrogen by 10% and single phosphorus by 10%. Combined nitrogen and phosphorus may be reflected in yield increase better than single treatments because these elements may affect the intake of each other. Whitelaw et al. (1997) reported that combined treatment of phosphate-solubilizing and nitrogen-fixing bacteria increased P intake and promoted plant growth. Çakmakçı (2007) reported higher bacteria efficiency for combined inoculations. In the present study, meaningful yield increases were observed with combined *B. megaterium*, *S. maltophilia*, and *R. pickettii* treatments. Microorganisms may cause different solubility and plants may intake plant nutrients more effectively. Significant differences were observed between single *S. maltophilia* and *R. pickettii* microorganism treatments and the control treatment. Compared to *B. megaterium*, a higher average value was observed. These findings revealed that *S. maltophilia* and *R. pickettii* were as effective as *B. megaterium* in P and N fixation. Similarly, Kundu and Gaur (1984) observed increased N and P intakes with phosphate-solubilizing bacteria and *Azotobacter* incubation. Belimov et al. (1995) reported significantly higher kernel yields in barley with combined phosphate-solubilizing *Agrobacterium radiobacter* and nitrogen-fixing *Azospirillum lipoferum* than single treatments of each. Çakmakçı (2005) also reported improved N and P intake with combined inoculations. Şahin et al. (2004) reported 11.9%–12.4% yield increase in sugar beet and 7.4%–9.3% in barley with dual inoculation of phosphate-solubilizing and N-fixing bacteria, and 12.7% yield increase in sugar beet and 9.3% in barley with triple inoculations. Belimov et al. (1995) also reported improved plant nutrient balance with combined treatments of phosphate-solubilizing and nitrogen-fixing bacteria. Phosphate-solubilizing *Bacillus* species promote growth through P nutrition (Banik and Ninawe, 1988; Whitelaw et al., 1997) and increase intake of other elements (Biswas et al., 2000). Çakmakçı et al. (1999) reported that *B. polymyxa* and *B. megaterium* treatments increased kernel yield of barley respectively by 12.4% and 9.2% in sandy soils and by 17.1% and 7.8% in loamy soils, and combined inoculation increased yield by 17.0% in sandy and 19.4% in loamy soils.

Considering current agricultural practices and certain indicators, it is impossible to mention the sustainability of these practices. Chemical fertilizers are among such unsustainable practices. Because of increasing mineral fertilizer costs and negative environmental impacts of these fertilizers, the interest in biological nitrogen fixation is increasing within the scope of sustainable agriculture (without significant yield losses). Therefore, effects of chemical fertilizer treatments were compared with phosphorus-solubilizing and nitrogen-fixing bacteria treatments on wheat yield and quality parameters were compared in the present study. Significant differences were observed among treatments with regard to all parameters. Except for harvest index, the highest values of all parameters were observed in either single N or N + P treatments. However, compared to the control treatment, single, dual, or triple bacteria combinations yielded significant increases in grain filling period, number of spikes per square meter, number of kernel per spike, 1000-kernel weight, biological yield, grain yield, grain protein ratio, plant flowering, and physiological maturity protein ratios. Such increases are especially distinctive in kernel yield (57% increase in kernel yield with M-13 + 73 + 82 triple bacteria combination) and in grain protein ratio (19.15%, 15.96%, and 14.1% increases with 73, 82, and M-13 + 73 bacteria treatments; 20.6%–32.4% increase in plant protein ratio in the heading period; 19% increase in plant protein ratio in the physiological maturity period). Based on these findings and considering the triple bacteria combination with regard to yield and protein ratios, it may be concluded that such treatments may reduce the amount of fertilizer to be used by 20%. Among the dual bacteria treatments, 73 + 82 and 82 + M-13 were found to be advantageous with regard to grain filling period, 73 + M-13 with regard to 1000-kernel weight and kernel protein ratio, and M-13 + 73 and M-13 + 82 with regard to plant protein ratio in the physiological maturity period. Combining bacteria strains with chemical fertilizers may be a missing part of the present study. Dual or triple combinations of bacteria with recommended or reduced doses of chemical fertilizers might have yielded better results. Therefore, such combinations may be recommended for future studies. It was concluded in the present study that biological fertilization may be an alternative for mineral fertilization. It is impossible to eliminate nitrogen totally from fields, but it was found beneficial to investigate the effects of microbial fertilization with small doses of mineral fertilizers.

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