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QUANTITATIVE AND QUALITATIVE RESPONSE OF WHEAT TO *PSEUDOMONAS FLUORESCENS* RHIZOBACTERIA APPLICATION

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ABSTRACT

Rhizobacterial application is efficient approach and process focusing less chemical fertilizers for increasing soil fertility and nutritional value of grains for different crops. This study investigated the effect of *Pseudomonas fluorescens* rhizobacterial application on the yield, yield components, grain quality and content of nutrition elements in wheat and in soil. Experiments were designed according to randomized complete block design with four replications in environmental conditions of Diyarbakir province, Turkey, during 2014 and 2015 growing seasons. Four treatments of *Pseudomonas fluorescens* rhizobacteria (control, seed inoculation, foliar application, seed + foliar) were used to investigate their effects on various aspects of wheat crop. Results of present study indicated that *P. fluorescens* rhizobacteria applications significantly ($P \leq 0.05$) affected grain yield, Al, K, Mg, and Zn content in grain, total N, and available P, K, Fe, and Mn elements in soil. The maximum grain yield was obtained from the combined application of seed inoculation + foliar application and showed 7% higher yield than in the control. Study indicated that application of rhizobacteria has positive effect on soil productivity, yield and nutritional quality of grains.

Keywords: Wheat, *Pseudomonas fluorescens,* rhizobacteria, seed inoculation, foliar application

INTRODUCTION

At present wheat occupies third position in world food production after rice and corn and almost two thirds of its production is used for human consumption. (Monneveux *et al.,* 2012). Along with the increase in wheat demand, inorganic fertilizers and chemical drugs (fungicide, insecticide, bactericide etc.) have brought about imbalanced and excessive use in agricultural production each passing day. These chemicals result in a high cost in agricultural production; cause accumulation of some toxic substances in water, soil, and food products, and also cause serious health problems in living organisms feeding on these crops. Therefore, in recent years biological applications (beneficial microorganisms) have gained importance in providing agricultural sustainability instead of chemical inputs to increase the resistance of plants against biotic and abiotic stress conditions, and to improve plant growth and yield. (Kıdoğlu *et al.,* 2008).

Microorganisms present in and around the plant root system are called as plant growth promoting bacteria (PGPR) playing a key role in increasing vegetative and generative development in plants (vegetables, fruits, ornamental plants, some trees, grains, etc.) and also provides protection against many factors that cause diseases in plants by promoting natural resistance (Güneş *et al.,* 2005). PGPRs generally play their role in plant development by colonizing in root system and keep the harmful rhizosphere microorganisms under pressure. These rhizobacteria may affect plant development by either producing growth hormones or changing the microbial balance in rhizosphere in favor of beneficial microorganisms directly or by organizing mineral substances' rate indirectly. Especially *Pseudomonas* and *Bacillus* stand out for having very well antagonistic aspects as well as their stimulant effects on plants development. (Ryu *et al.,* 2011).

In recent years, PGPR bacteria have been used for different plants to improve the plant growth and soil organic matter level. In the research conducted with *Bacillus* species, significant growth have been recorded in the yield of rough rice (Sharma *et al.,* 2013) and corn (Pal, 1998). Many researchers reported effect of *P. fluorescens* inoculation on t root dry weight of the spring wheat (Hassan and Bano, 2015; Walley and Germida, 1997). Similar results obtained for yield of the applied and unapplied conditions of *P. fluorescens* in corn (Shaharoona *et al.,* 2006). Bashan and de Bashan (2005) reported that *P. fluorescens* applications reduce plant depression of corn under different salt concentrations. Afzal *et al.* (2014) reported that *P. Fluorescens* increases tillering in wheat, plant height, number of spikes per plant, number of spikelets in a spike, 1000-grain weight, grain set yield, and phosphor concentration.

Wheat quality properties are mostly related with protein content in grain. Uptake of nitrogen highly needs free nitrogen in soil and genetic potential of varieties. High nitrogen uptake by plants has positive effect on protein content of grain and other quality traits. In

consideration of these findings; the present study has been conducted with the purpose of determining the effect of *P. fluorescens* rhizobacteria on yield and yield components of wheat, ii) grain quality and iii) some macro-micro nutrients content in seed and soil.

MATERIALS AND METHODS

Soil and climate conditions of the experimental area: Experiments were performed at experimental field of International Agricultural Research and Training Center, Diyarbakir GAP during 2014 and 2015 under rainfall conditions.

The testing site has been determined to have clay-loamy soil, medium salty by means of total salt value (0.400 %), low by means of organic substance (1.66%), alkaline by means of soil reaction (pH: 8.07), and medium level by means of $CaCO_3$ content (7.93 %). Diyarbakir province, where the research was conducted, reflects terrestrial climate. Summers are very hot and dry, while winters are cold with less precipitation. The average temperature of Diyarbakir in the years, in which the experiments were conducted during the wheat vegetation period, was respectively 20.0 and 14.7 °C, amount of rainfall was 356.7 and 583.8 mm. Meteorological parameters of Diyarbakır province for 2013-14 and 2014-15 wheat growing seasons are presented in Table 1.

Table 1. Meteorological parameters of Diyarbakır province for 2013-14 and 2014-15 wheat-growing seasons

Years	Climate parameters	Months									
		September	October	November	December	January	February	March	April	May	June
2013-2014	Max. Temp.	32.2	24.2	14.5	10.2	7.6	10.6	14.2	19.2	27.1	34.4
	Min. Temp.	16.5	11	2.9	3.7	-2.2	1.4	2.6	5.5	10.3	15.9
	Rainfall	27.4	34.2	97.6	73.6	64.6	55.2	127	48.6	48.2	7.4
2014-2015	Max. Temp.	32.1	25.0	17.6	1.4	9.2	13.4	16.8	22.0	28.1	34.1
	Min. Temp.	15.9	9.0	6.1	-7.1	-1.0	-1.0	4.9	6.9	11.1	17.6
	Rainfall	0	0	54	50.4	43	38.6	60.6	39.9	48.8	21.4

Materials: Dinç cultivar (bread wheat, white grain, semi-hard and spring bread wheat variety) developed by the GAP International Agricultural Research Institute was used as plant material in the research. A water-based microbial preparation (esbioful) containing 10^8kob/g *Pseudomonas fluorescens* bacterium was used as PGPR.

Methods: Experiments were established according to randomized complete block design with four replications. Crop was sown in six row plots measuring an area of 12 m^2, and seed rate was 450 seeds per square meter. In both years, sowing was done in November 15th. In the experiment, *P. Fluorescens* bacteria was applied in four different applications (control (T1): non-rhizobacteria, seed inoculation (T2): 2000 ml esbioful per one tone seeds, foliar application (T3): 1500 ml esbioful ha^{-1}at bolting period, seed inoculation + foliar application (T4). In this study; yield, quality, agronomic characteristics and some nutrient contents in seed and soil were investigated after harvest. Agronomical observations were taken from 10 plants randomly selected in each treatment plots. Grain quality analysis (hectoliter, protein ratio, sedimentation, and wet gluten) was performed with Perten 9500 brand Near Infrared Transmission (NIT) analysis device that operates by near infrared transmission method on grain sample obtained from each treatment plots after harvest. Harvesting was done between June 15th – 30th for both years. Macro and microelements in grains were determined by micro and macro elements ICP-OES method (Thermo Smart D wet combustion method) after performing cleaning, washing, drying, grinding, and final drying operations according to Kacar, (1972). Se (Selenium) was measured by hydride system. Analysis of soil samples of experiment plots were made after harvest. Soil samples were obtained as to represent 0-30 cm soil depth plots in accordance to principles reported by Jackson (1967). Organic substances were determined by dry combustion method (Kacar, 1994); total N by Kjeldhal method; K, Ca, Mg, Zn, Cu, Mn, Fe by wet combustion method (Nitric-Pecloric Acid) (Kacar, 1972) and available P by Barton method with spectrophotometric device.

Variance analysis of the data obtained t was performed in JMP 5.1 statistical package by performing combined analysis over the years. LSD multi-comparison test was used in revealing effective differences. GGE biplot analysis was performed by GENSTAT 12th statistical program used to calculate first and second principal components (PC1 and PC2) for elements in soil.

RESULTS AND DISCUSSION

Yield and yield components: The application of *P. fluorescens* rhizobacteria was found to have a significant effect on grain yield, number of days to heading, plant height, number of spikes per square meter and number of grains per spike while the effect of this application was not statistically significant for the number of days to maturity, spike length, number of spikelet per spike, grain

weight per spike, biological weight, and harvest index (Table 2). The ranges of the values were as follows: 7793.4 to 8382.2 kg $ha^{-1}$for grain yield, 114.7 to 117.2 days for heading days, 96.2 to 100.7 cm for plant height, 602.5 to 635.0 spikes per square meter, and 53.0 to 63.5 spikelet for spikelet per spike. The lowest number of days to heading was obtained from T2 and T4 (114.7 days); the highest grain yield and plant height were observed in T4 (8382.2 kg ha^{-1} and 100.7 cm, respectively), and the highest number of spikes per square meter and spikelet per spike were found in T3 (Table 3). This showed that there were no statistically significant differences between the T2, T3, and T4 applications, which all involved the use of *P. fluorescens* rhizobacteria; however, the results of these applications were significantly different from those of T1, in which rhizobacteria were not used (Table 3). For several traits that were examined, T2, T3, and T4 were in the same group while T1 was found to produce different results (Table 3). Although the values of biological weight, days to maturity, spike length, number of spikelet per spike, and grain weight per spike did not significantly change between the applications, they were generally higher in rhizobacteria applications (T2, T3, and T4) compared to the control (T1) application (Table 3).These results indicated that rhizobacteria has positive effects on examined traits. Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth using a wide variety of mechanisms (Ashrafuzzaman *et al., 2009*). Our results are similar to those of Vessey, (2003) who reported that rhizosphere, *Pseudomonas* bacteria, *P. fluorescens* and *P. putida* in particular, are commonly seen in soil, and increase the yield in several plants. In addition, it has been found that *Pseudomonas* sp. isolates increase the grain and straw yield in wheat (Shaharoona *et al.,* 2007), and corn seeds inoculated with *P. fluorescens* significantly improved plant height, 100-grain weight, number of grain per cob, and grain yield (Nezarat and Gholami, 2009). Hassan and Bano, (2015) applied tryptophan combined with *Pseudomonas moraviensis* and *Bacillus cereus* to wheat and reported that this application increase number of plants per square meter, spike length, number of spikelet per spike, and grain weight. These results support those obtained from the present study. Yousefi and Bargezar, (2014) reported that inoculation of *Pseudomonas* and *Azotobacter* affect positively wheat yield and yield components. Hasanzadeh *et al.* (2007) reported that bacterial inoculation increased dry matter accumulation in wheat and sorghum.

Grain quality: According to the results, there were no statistically significant differences between the applications in terms of these quality traits (Table 2). However, apart from the hectoliter weight, the values related to all the remaining quality traits were found to be higher in *P. fluorescens* rhizobacteria applications (T2, T3 and T4) than in the control (T1) application (Table 3). The highest thousand kernel weight was obtained from T3 (36.44 g) and the highest values of protein content, sedimentation, and wet gluten were observed in T4 (11.1%, 26.5, and 22.7, respectively), while the highest hectoliter control treatment but all treatments joined the same statistical group (Table 3). In general, *P. fluorescens* are usually referred to as Plant-Growth Promoting Rhizobacteria, which can affect yield, yield component and quality for several plants. Our results also indicated that *P. fluorescens* application had positive affects almost for all examined traits.

Our results concerning the thousand kernel weight of wheat following *P. fluorescens* applications are consistent with those reported by Egamberdieva (2010) that the highest protein content, 1000 kernel weight and hectoliter weight values obtained in *P. fluorescens* rhizobacteria inoculation plots compared to control plots in wheat cultivars. Rhizobacteria increase free nitrogen, macro and microelements in soil resulted with higher elements uptake by plants. Many reports indicated that bacteria such as *azobacter*, *azospirillum*, *pseudomonas* and *bacillus* are involved in nitrogen fixation and they are able to increase nitrogen in soil, and also have potential to increase available nutrient elements for plants (Taurian *et al.,* 2010; Nezarat and Gholami 2009). The present study indicated that obtaining highest grain protein content in T4 (seed inoculation + foliar application) could be related to *P. fluorescens* rhizobacteria inoculation.

Table 2. Analysis of variance of measured agronomic and quality parameters of wheat

Source	Df	Heading days	Maturating days	Plant Height (cm)	Spike number (m^2)	Spike length (cm)	Spikelet number (per spike)	Grain number (per spike)	Kernel weight (per spike)
Year	1	128.00**	32.00**	288.32*	20808.00**	1.280^{ns}	46.08*	327.68**	0.32^{ns}
R(Y)	8	12.50	13.50	199.45	2162.50	2.42	17.93	245.01	0.15
T	3	41.50*	18.52*	87.24*	7262.50*	3.82^{ns}	5.74^{ns}	557.21*	0.52^{ns}
Y X T	9	25.35^{ns}	5.34^{ns}	45.30^{ns}	2298.42^{ns}	1.12^{ns}	9.26^{ns}	125.38 ns	0.16^{ns}
Error	18	9.50	23.45	76.28	9576.64	8.89	31.48	412.61	0.12
CV		**2.9**	**1.8**	**2.09**	**2.57**	**7.28**	**6.66**	**8.13**	**13.57**

Table 2. (Continue) Analysis of variance of measured agronomic and quality parameters of wheat.

Source	Df	Harvest Index	Biological yield (kg ha^{-1})	Grain yield (kg ha^{-1})	1000- grain weight (g)	Hecto liter	Protein content (%)	Sedimen tation	Wet gluten
Year	1	103.68**	1280000.0*	39762.00**	5.12 ^{ns}	46.08**	1.28 ^{ns}	392.00*	128.00**
Rep (Y)	6	3.09	341710.1	3499.67	44.02	0.73	8.50	610.50	44.78
Treatments	3	23.26 ^{ns}	337751.7 ^{ns}	14620.75*	5.52 ^{ns}	0.21 ^{ns}	0.63 ^{ns}	40.52 ^{ns}	3.29^{ns}
Y X T	3	4.46 ^{ns}	225402.0 ^{ns}		5.34 ^{ns}	0.65 ^{ns}	0.25 ^{ns}	20.38 ^{ns}	3.85^{ns}
Error	18	70.34	994001.7	9576.64	37.88	1.50	19.13	10.72	91.74
CV		**4.55**	**11.21**	**2.84**	**4.04**	**0.34**	**9.44**	**12.72**	**10.28**

* and ** are respectively significant at P ≤ 0.05 and P ≤ 0.01, ns: not- significant, Y: Year; R: Replication; T: Treatment.

Table 3. The effects of applying *Pseudomonas fluorescens* rhizobacteria applications in wheat to yield, yield components, and quality aspects

Applications	DH	DM	PH	NSPS	SL	SPS	GPS	GWS	HI
T1	116.7 a	182.5	96.2 b	602.5 b	9.12	19.20	53.0 b	2.33	42.03
T2	114.7 b	181.3	97.5 ab	629.2 a	9.61	19.80	57.0 ab	2.43	43.81
T3	115.2 b	181.8	98.5 ab	635.0 a	9.95	20.15	63.5 a	2.68	43.87
T4	114.7 b	181.0	100.7 a	633.7 a	9.97	20.30	62.0 a	2.48	44.22
Average	115.8	181.9	98.2	625.1	9.66	19.86	58.9	2.48	43.48
CV (%)	2.9	1.8	2.09	2.57	7.28	6.66	8.13	13.57	4.55
LSD	1.16*	Ns	3.29*	25.47*	Ns	Ns	7.66*	ns	Ns
Applications	**BY**	**GY**	**1000 KW**	**HL**	**PC**	**SD**	**WG**		
T1	19906.2	7793.1 b	35.31	84.85	10.7	23.5	21.9		
T2	20229.0	8149.2 ab	36.44	84.78	10.9	24.8	22.2		
T3	21167.3	8203.3 a	36.13	84.75	11.0	25.8	22.5		
T4	22552.1	8382.6 a	35.81	84.63	11.1	26.5	22.7		
Average	20963.7	8132.1	35.92	84.75	10.9	25.1	22.3		
CV (%)	11.21	2.84	4.04	0.34	9.44	12.72	10.28		
LSD	Ns	369.0*	ns	Ns	Ns	ns	Ns		

Days to Heading (DH), Days to Maturity (DM), Plant Height (PH), Number of Spike per Square Meter (NSPS), Spike Length (SL), Number of Spikelet's per Spike (SPS), Number of Grain per Spike (GPS), Grain Weight per Spike (GWS), Harvest Index (HI), Biological Yield (BY), Grain Yield (GY), 1000 Kernel Weight (1000 KW), Hectoliter (HL), Protein Content (PC), Sedimentation (S) and Wet Gluten (WG), * and ** are respectively significant at the levels of P<0.05 and P<0.01; NS: not significant. Differences between the averages shown with same letters are non-significant at a level of P<0.05.

Macro and micro nutrient content of seeds: In this study, the *P. fluorescens* rhizobacteria applications were found to have a statistically significant effect on the Al, K, Mg, and Zn content of wheat (p< 0.05) whereas no statistical significance was observed in the percentages of Ca, Cr, Cu, Fe, Mn, P, and Se (Table 4). In the statistically significant group, Al ranged from 11.47 to 24.29 ppm, K from 508.65 to 2604.10 ppm, Mg from 811.8 to 918.7 ppm, and Zn from 10.86 to 13.40 ppm (Table 5). The highest Al, Mg, and Zn values were obtained from the T4 application, and the highest K was detected in T3. Concerning the elements that were not found to be statistically significant, the grain content of Ca, Cu, Fe, P, and Se was generally higher following the *P. fluorescens* rhizobacteria applications compared to the control application. These results could be related to potential of rhizobacteria that increase N fixing and uptake of N, P, K, Ca, manganese (Mn), zinc (Zn) and Fe. Similar results were reported by researchers for different crops, for example, increased Fe, Mn, Zn, and Cu in barley (Çakmakçı *et al.,* 2007a; Shaharoona *et al.,* 2007) and increased macro nutrients (N, P, K, Ca, Mg, and S) and micro nutrients (Fe, Mn, Zn, and Cu) in wheat (Turan *et al.,* 2010). The results of the present study are consistent with those reported in the literature. Sharma *et al.* (2013) reported that *P. Fluorescens, P. putida* and *A. lipoferum* bacteria have similar impact on iron (Fe) content of rice and increase significantly microelement contents of rice. Vaid *et al.* (2013) found that when *P. fluorescens* rhizobacteria application used individually increased the Zn concentration in wheat grains and in straw significantly over the control.

Table 4. Analysis of variance of measured micro and macro elements of wheat

Variable	Df	Al	Ca	Cr	Cu	Fe	K
Year	1	6.00^{ns}	5400.0**	0.54*	0.90^{ns}	26.46*	18438.23**
Rep (Y)	8	31.98	1388.70	0.07	1.82	35.18	4762.73
Treatments	3	592.49*	352.74^{ns}	0.04^{ns}	2.49^{ns}	6.01^{ns}	44410.77*
Y X T	9	24.46^{ns}	126.32^{ns}	0.02^{ns}	0.77^{ns}	5.38^{ns}	375.106^{ns}
Error	18	109.59	1655.48	0.05	3.120	49.00	9704.24
CV		**14.04**	**12.5**	**13.52**	**8.89**	**6.81**	**1.39**
Variable	**Df**	**Mg**	**Mn**	**P**	**Se**	**Zn**	
Year	1	15000.0**	6399.78**	2400.0**	0.54*	31.74**	
Rep (Y)	8	5771.1	35.51	3028.9	0.03	2.61	
Treatments	3	35572.4*	28.49*	7208.7^{ns}	0.23^{ns}	21.43*	
Y X T	9	4245.3^{ns}	2345*	66.8^{ns}	0.04^{ns}	3.18^{ns}	
Error	18	10910.8	13.06	2052.5	0.13	4.12	
CV		**4.01**	**7.23**	**5.26**	**13.92**	**6.14**	

Table 5. Effects of *pseudomonas fluorescens* rhizobacteria application in wheat on some macro and micro element contents in grains

Applications	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	P	Se	Zn
T1	11.47 b	111.90	0.432	2.49	29.01	2508.65 b	811.8 b	40.53	361.79	0.468	10.86 b
T2	20.24 ab	120.63	0.383	2.97	30.28	2599.88 a	863.4 ab	35.58	405.39	0.725	12.64 a
T3	22.80 a	118.35	0.417	2.63	29.42	2604.10 a	847.9 ab	35.50	389.30	0.683	11.80 ab
T4	24.29 a	121.84	0.323	2.63	30.03	2514.55 ab	918.7 a	35.42	402.98	0.605	13.40 a
Average	19.70	118.18	0.389	2.68	29.69	2556.80	860.45	36.76	389.87	0.620	12.18
CV (%)	14.04	12.5	13.52	8.89	6.81	1.39	4.01	7.23	5.26	13.92	6.14
LSD	9.67*	Ns	Ns	Ns	ns	90.83*	66.65*	ns	ns	ns	1.83*

* and ** are respectively significant at the levels of P<0.05 and P<0.01; ns: not significant.
Differences between the averages shown with same letters are insignificant at a level of P<0.05.

Effect of the Applications on Soil Elements: The results showed that compared to the control application, the T2, T3, and T4 applications that involved *P. fluorescens* rhizobacteria inoculation had a higher positive effect on the amount of the N, P, K, Mg, Fe, Mn, Zn, and Cu elements and a negative effect on Ca. The organic matter content and total N of soil as well as the K and Mg elements were found to be higher in T2 whereas T3 was prominent in terms of P and Zn, and T4 resulted in the highest Fe, Cu, and Mn values. Similarly, Hassan and Bano, (2015) reported that inoculation of wheat with *P. moraviensis* and *B. cereus* rhizobacteria increased the N, P, K, and Mg nutrients, and organic matter content of soil. There are many reports indicated that *Pseudomonas fluorescens and Bacillus subtilis* are able to facilitating nutrient uptake and increase available nutrients such as N, P, K, Mg, Fe, Mn, Zn and organic matter in soil, this provides using lower chemical fertilizer and contributes to protecting of ecological balance in soil (Idris *et al.,* 2009; Nezarat and Gholami, 2009).

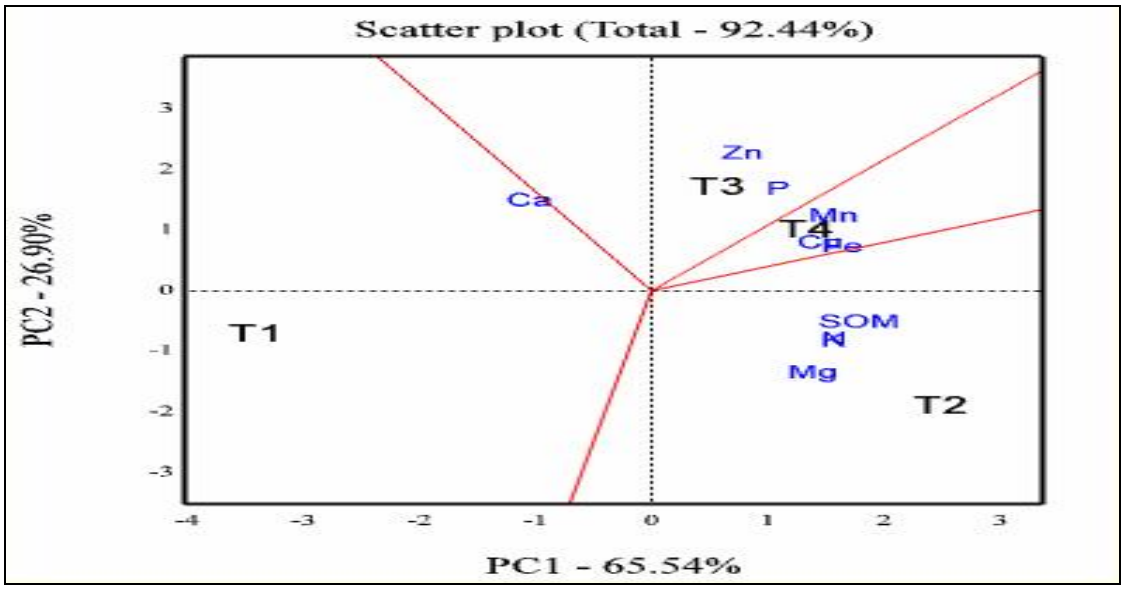

Figure 1. Effect of *Pseudomonasfluorescens* rhizobacteria applications on organic substance ratio in the soil and on some obtainable element amounts

Conclusions: This study showed that the two different applications of *P. fluorescens* rhizobacteria (PGPR group bacteria) namely seed inoculation and foliar application were effective in promoting plant growth, and both were found to be superior to the control application. The *P. fluorescens* rhizobacteria application had a positive effect on wheat grain yield, yield components, quality and certain nutrients in grain and soil.

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