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Response of Yield and Yield Components of Rice (*Oryza sativa* L.) to *Pseudomonas flouresence* and *Azospirillum lipoferum* under Different Nitrogen Levels

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Abstract: In order to study the effect of *Pseudomonas flouresence* and *Azospirillum lipoferum* isolates on yield and yield components of rice (*Oryza sativa* L.) cultivar Tarom Daylamani, an experiment was conducted in Mazandaran province, city Neka in 1388. A split factorial experiment under complete randomized block design with three replications was used for this research. The first factor was pure bacterial isolates *Azospirillum lipoferum* (A) and *Pseudomonas flouresence* (B) and the combination of these two types of bacteria (A1B1) and the second factor included four levels of nitrogen (25, 50, 75, 100 kg ha) of urea. The analysis of variance showed that the effect of nitrogen fertilizer and bacteria had no significant difference on 1000 seeds weight, number of clusters (m2) and number of grains per panicle. The effect of fertilizers, bacteria *Azospirillum lipoferum* and *Pseudomonas flouresence* on rice yield showed that application of 100 kg of nitrogen with *Pseudomonas* and without *Azospirillum* had the highest yield of 5733 kg/ ha. Results of analysis of variance showed that fertilizers with bacteria, *Pseudomonas flouresence* and *Azospirillum lipoferum* had a significant effect on harvest index at $P<0.01$ level. Data also indicated that yield had the highest positive correlation with panicle and harvest index. The most important aim of this research was to reduce consumption of chemical fertilizers by using biological fertilizers which could lead to an effective step in preserving the environment.

Key words: Rice · *Pseudomonas flouresence* · *Azospirillum lipoferum* · Nitrogen fertilizer · Seed yield · Yield components

INTRODUCTION

Rice *(Oryza sativa)* is the major food crop of nearly half of the world's population. Total rice-cropped area and rough rice production in the world was 147.14 million hectares and 576.28 million tons, respectively in 2002 [International Rice Research Institute (IRRI) 2003]. Rice plants require large amounts of mineral nutrients including N for their growth, development and grain production. Rice crops remove 16-17 kg N for the production of each ton of rough rice including straw [1]. The indiscriminate use of fertilizers containing cadmium have adverse effects on yield increment and public health [2], therefore using bacteria genus *Pseudomonas* is useful. Solubility of phosphorus is an important features as growth stimulant for this bacteria [3, 4]. Another useful role of stimulating growth bacteria for plants is to reduce or eliminate the harmful effects of pathogen agents, through the phenomenon of Induced Systemic Resistance (ISR) in plants [5]. Salicylic acid produced by plant growth stimulants bacteria could induce systemic resistance phenomenon in the rhizospher [5]. Indiscriminate use of nitrogen fertilizers cause soil pollution and groundwater nitrate pollution, especially in the northern parts of the country. *Azospirillum* is the most famous microorganism that can produce colonies in the rhizospher around cereals roots and lead to nitrogen fixation. The increment of soil nitrogen amount increases the populations of some bacteria in the rhizospher of some plants, but decreases the number of bacteria involved in nitrogen fixation [6]. The most important aim of this study is to reduce chemical fertilizers consumption by using biological fertilizers

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which could be an effective step in preserving the environment. Plant growth promoting rhizobacteria (PGPR) are group of soil bacteria that actively colonize plant roots and increase plant growth and yield [7, 8]. PGPR belong to a range of genera, including *Pseudomonas, Azotobacter, Azospirillum, Bacillus* etc. [9, 10]. The mechanisms by which PGPR can promote plant growth are not fully understood, but are thought to include symbiotic nitrogen fixation [11], the ability to produce phytohormones [12], solubilization of phosphates [13] and production of ACC deaminase [14]. Significant increase in growth and yield of important agronomical crops in response to inoculation with PGPR have been demonstrated by many researchers [9, 15, 16]. In last few decades a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Entrobacter*, *Alcaligens*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia* have beenreported to enhance plant growth [7, 17, 18]. A substantial amount of the urea-N is lost through different mechanisms including ammonia volatilisation, denitrification and leaching losses, causing environmental pollution problems [19]. Utilization of biological nitrogen fixation (BNF) technology can decrease the use of urea-N, prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent [20]. Yield increases in rice due to inoculation of *Azospirillum* and *Azotobacter* are reported to be in the 5-60% range [21]. Among the plant growth stimulating bacteria, genus *Pseudomonas* is significantly important due to its wide distribution in the soil, ability of colonization in many plants rhizospher and production of a wide range of metabolites [22]. These bacteria have a wide range of plant growth stimulants traits such as producing auxin, ACC deaminase enzyme, phosphate solubility, siderophore, salicylic acid, hydrogen cyanide and chitinase which are directly or indirectly involved plant growth in increasment [23, 24]. Farah - Ahmed *et al*. [25] showed that growth rate and root elongation in seed germination of *Sesbania aculeate* and *Vigna radiate* inoculated with *Pseudomonas* increased due to the production of indole -3 - acetic acid. Benizri *et al*. [26] reported that *Pseudomonas flouresence* M. 1,3 is able to produce auxin, plays an important role in plant growth and increase plant production. Egamberdieva [12] reported that inoculation of winter wheat with *Pseudomonas flouresence* strain 12 RSIA,increased root and shoot growth and absorption of nitrogen, phosphorus and potassium. However, most of the worlds rice farms soil are nitrogen deficient therefore so N fertilizer applications are required to meet the crops N demand. Generally, urea is applied as the N source for rice production. But the efficiency of adding urea is very low, usually 30-40% and even lower [27, 28]. NH_3 volatilization and denitrification cause atmospheric pollution through the production of greenhouse gases like N_2O and NH_3 [29]. In addition to these environmental problems, the long-term use of urea depletes the soil organic matter. These problems are of great concern to soil and environmental scientists around the world. Alternate source of N should be applied to minimize these problems if possible. Biological N fixation (BNF) technology can play an important role in substituting for commercially available N fertilizer use in rice culture, thus reducing these environmental problems to some extent. Use of biofertilizers can prevent the depletion of the soil organic matter [30]. Rice crops are grown in both wetland and upland cultures. However about 85% of the total rice-cropped area is under wetland culture. In upland culture aerobic bacteria can fix atmospheric N while in wetland culture both aerobic and anaerobic bacteria can fix N [31]. Other biological N fixers used in rice culture are *Azospirillum, Herbaspirillum and Burkholderia* [32]. *Azospirillum* is a heterotrophic bacterium capable of fixing atmospheric N [33]. This organism grows in the rhizospher of graminaceous plants. It can also penetrate the root to grow intercellularly [34]. Both *Azospirillum lipoferum* and *A. brasilence* have been isolated from roots and stems of rice plants [35]. Investigations at the IRRI revealed that *Azospirillum* constitutes about 1% of the total aerobic heterotrophs and about 85% of the *Azospirillum* isolates belong to *A. lipoferum* indicating its preferential colonization for rice plants [36]. *Azospirillum* inoculation can increase PO_4^{-3} and NH_4^+ uptake by rice plants [37]. This species can also increase the height and tiller number of rice plants [38]. *Azospirillum* inoculation increased the rice yield significantly by 1.6-10.5 g $plant^{-1}$ (32-81% increase) in greenhouse conditions [32]. However, in field conditions, the estimated yield increase was around 1.8 t ha^{-1} (22% increase) as reported by Balandreau [39].

MATERIALS AND METHODS

This field research was conducted in Mazandaran province, city Neka with 36 degree north latitude and 53 degrees east and 12 m below sea level with the rice cultivar Tarom Daylamani during 2009. A split factorial arrangement in randomized complete block design with three replications was employed. The first factor was pure isolates of bacteria: *Pseudomonas flouresence* (B), *Azospirillum lipoferum* (A) and a combination treatment of these two types of bacteria (A1B1) and the second factor included four levels of Nitrogen fertilizer (25, 50, 75, 100 kg ha^{-1}) of urea. The experiment field soil was silty clay with pH = 8. Based on soil test results, 50 kg ha^{-1} of each super phosphate triple and potassium sulfate was applied before planting and 50 kg of potassium chloride fertilizer at the end of tiller stage and nitrogen fertilizers were applied based on research objectives. *Pseudomonas* and *Azospirillum* bacteria treatments were assigned to main plots and four levels of nitrogen fertilizer were assigned to sub-plots. For bacteria inoculation, Roots of seedlings were inoculated with bacteria for at least 12 hours. After inoculation of seedlings in late April, the seedling were moved to the main land in June. Transplantation distance was 25 × 25 cm between each pile and each pile consisted of three transplant (germ). Nitrogen consumption sources were urea used in three important growth stages. Weed control was applied seven days after transplantation as mechanical and without use of chemical herbicide and been repeated 20 days after the first time. Due to bacteria sensitivity, no chemicals was used for weed and pest control in this experiment. 10 plants from each experimental plot were randomly selected to measure the characteristics for each trait. For nutrient analysis leaf samples were collected at the stage of 50% flowering. At harvest stage, grain yield and yield components were determined. Data analysis of variance was analyzed with MSTAT-C statistical software and using Duncan's Multiple Range Test (DMRT) at 5% probability level for means comparison.

RESULTS AND DISCUSSION

Grain Yield: Table 1 and Tables 2, 3, 4 and 5 shows analysis of variance and the results of data analysis for different bacteria and nitrogen amounts on rice yield respectively. According to the analysis of variance results grain yield had been significantly influenced by bacteria *Pseudomonas flouresence* at 1% probability level(Table 1). Nitrogen fertilizer treatments had a significant effect on grain yield at 5% probability level and the combined treatment of *Pseudomonas flouresence* and *Azospirillum lipoferum* also had a significant effect at 1% probability level. Plant growth promoting rhizobacteria (PGPR) are group of soil bacteria that actively colonize plant roots and increase plant growth and yield [7, 8]. Significant increase in growth and yield of agronomical important crops in response to inoculation with PGPR have been demonstrated by Bashan *et al*. [9], Asghar *et al*. [15] and Biswas *et al*. [16]. Result of analysis of variance showed that the effect of nitrogen and bacteria (separately and in combination) on 1000 seeds weight and number of grains per panicle was not significant (Table 1). The interaction between nitrogen and bacteria *Pseudomonas flouresence* and *Azospirillum lipoferum* on harvest index had a significant effect at 1% probability level. Nitrogen treatment also showed a significant effect on number of clusters at 1% probability level (Table 1). Wheat, sorghum and maize seeds inoculated with the bacterium *Pseudomonas aeruginosa* in America caused a 10 to 30 percent yield increasment [40]. The interaction between different levels of nitrogen and bacteria showed that highest yield (4867 kg ha^{-1}) was produced with the combination of 100 kg of nitrogen fertilizer treatment with

Table 1: Analysis of variance of measured traits

		Mean Square				
SOV	df	Grain yield	Numbers of panicles/m^2	Harvest index	1000 seed weight g	Numbers of grains per panicle
rep	2	4155833.3	397.94	20.06	7.98	1681.29
nit	3	7849722.2*	1156.36**	45.21^{ns}	34.73**	311.03^{ns}
error	6	1024722.2	22.132	49.28	0.295	80.02
azo	1	140833.3^{ns}	12.0^{ns}	49.09^{ns}	4.88*	11.71^{ns}
nit×azo	3	136388.9^{ns}	125.61^{ns}	62.33^{ns}	1.38*	24.44^{ns}
pes	1	156408333.3**	270.75^{ns}	160.0^{ns}	32.84**	185.22^{ns}
nit×pes	3	54166.7^{ns}	3.58^{ns}	17.92^{ns}	1.89*	89.75^{ns}
azo×pes	1	9900833.3^{ns}	176.33^{ns}	649.39^{ns}	16.22**	114.73^{ns}
nit×azo×pes	3	234166.7^{ns}	127.39^{ns}	28.68**	1.51*	21.79^{ns}
error	24	443055.6	140.11	41.2	0.44	129.83
CV%	-	16.25	16.47	14.37	3.04	13.23

ns; nonsignificant; **, significant at p≤0.01; *, significant at p≤0.05. azo: *Azospirillum lipoferum*,,pse: *Psedumonas flouresence*, nit: Nitrogen.

Table 2: Mean comparison of interaction effect of Nitrogen fertilization and *Azospirillum* application

Treatment	Grain yield kg ha^{-1}	Numbers of panicles / m^2	Harvest index	1000 seed weight (g)	Numbers of grains / panicle
N_1A_1	2933^{B}	62.50^{B}	45.8^{AB}	19.27^{D}	79.86^{A}
N_1A_2	2867^{B}	60.17^{B}	38.34^{B}	20.33^{C}	80.0^{A}
N_2A_1	4267^{A}	69.0^{AB}	47.96^{A}	20.77^{C}	90.64^{A}
N_2A_2	4400^{A}	63.33^{B}	43.98^{AB}	21.97^{B}	91.42^{A}
N_3A_1	4500^{A}	72.83^{AB}	45.92^{AB}	22.12^{B}	86.99^{A}
N_3A_2	4467^{A}	82.0^{A}	46.71^{AB}	22.7^{B}	91.91^{A}
N_4A_1	4467^{A}	81.17^{A}	42.91^{AB}	23.98^{A}	85.0^{A}
N_4A_2	4867^{A}	84.0^{A}	45.55^{AB}	23.68^{A}	83.12^{A}

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$. N_1=25 kg/ha, N_2=50 kg/ha, N_3=75 kg/ha, N_4=100 kg/ha, A_1= without *Azospirillum* application, A_2= *Azospirillum* application.

Table 3: Mean comparison of interaction effect of Nitrogen fertilization and *Pseudomonas* application

Treatment	Grain yield kg ha^{-1}	Numbers of panicles / m^2	Harvest index	1000 seeds weight (g)	Numbers of grains / panicle
N_1B_1	2333^{C}	58.50^{E}	39.12^{B}	19.13^{E}	80.53^{B}
N_1B_2	3467^{B}	64.17^{CDE}	45.02^{AB}	20.47^{D}	79.33^{B}
N_2B_1	3800^{B}	63.33^{DE}	43.42^{AB}	20.70^{D}	96.67^{A}
N_2B_2	4867^{A}	69.0^{BCDE}	48.52^{A}	22.03^{C}	85.39^{AB}
N_3B_1	3967^{B}	75.67^{ABCD}	44.70^{AB}	21.85^{C}	88.78^{AB}
N_3B_2	5000^{A}	79.17^{ABC}	47.93^{A}	22.97^{B}	90.13^{AB}
N_4B_1	4000^{B}	80.50^{AB}	44.07^{AB}	22.42^{BC}	86.36^{AB}
N_4B_2	5333^{A}	84.67^{A}	44.46^{AB}	25.25^{A}	81.76^{AB}

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$. N_1=25 kg/ha, N_2=50 kg/ha, N_3=75 kg/ha, N_4=100 kg/ha, B_1= without *Pseudomonas* application, B_2= *Pseudomonas* application.

Table 4: Mean comparison of interaction effect of Nitrogen fertilization, *Azospirillum* application and *Pseudomonas* application

Treatment	Grain yield kg ha^{-1}	Numbers of panicles / m^2	Harvest index	1000 seeds weight (g)	Numbers of grains / panicle
$N_1A_1B_1$	1867^{I}	62.0^{BC}	36.87^{DE}	18.30^{J}	78.32^{A}
$N_1A_1B_2$	4000^{CDEFGH}	63.0^{ABC}	54.73^{A}	20.23^{HI}	81.40^{A}
$N_1A_2B_1$	2800^{HI}	55.0^{C}	41.38^{BCDE}	19.97^{HI}	82.73^{A}
$N_1A_2B_2$	2933^{GHI}	65.33^{ABC}	35.30^{E}	20.70^{GHI}	77.27^{A}
$N_2A_1B_1$	3467^{EFGH}	64.67^{ABC}	42.61^{ABCDE}	19.67^{I}	95.95^{A}
$N_2A_1B_2$	5067^{ABC}	77.33^{ABC}	53.31^{AB}	21.87^{DEFG}	85.33^{A}
$N_2A_2B_1$	4133^{CDEFG}	62.0^{BC}	44.23^{ABCDE}	21.73^{DEFG}	97.38^{A}
$N_2A_2B_2$	4667^{ABCDE}	64.67^{ABC}	43.72^{ABCDE}	22.20^{CDEF}	85.45^{A}
$N_3A_1B_1$	3533^{DEFGH}	65.67^{ABC}	41.16^{BCDE}	21.07^{FGH}	85.77^{A}
$N_3A_1B_2$	5467^{AB}	80.0^{AB}	50.67^{ABC}	23.17^{BC}	88.22^{A}
$N_3A_2B_1$	4400^{BCDEF}	85.67^{A}	48.23^{ABCD}	22.63^{CDE}	91.78^{A}
$N_3A_2B_2$	4533^{ABCDE}	78.33^{AB}	45.19^{ABCDE}	22.77^{CD}	92.03^{A}
$N_4A_1B_1$	3200^{FGH}	76.0^{ABC}	40.0^{CDE}	21.47^{EFG}	84.13^{A}
$N_4A_1B_2$	5733^{A}	86.33^{A}	45.97^{ABCDE}	26.50^{A}	85.87^{A}
$N_4A_2B_1$	4800^{ABCD}	85.0^{AB}	48.14^{ABCD}	23.37^{BC}	88.58^{A}
$N_4A_2B_2$	4933^{ABC}	83.0^{AB}	42.95^{ABCDE}	24.0^{B}	77.65^{A}

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$. N_1=25 kg/ha, N_2=50 kg/ha, N_3=75 kg/ha, N_4=100 kg/ha, A_1= without *Azospirillum* application, A_2= *Azospirillum* application, B_1= without *Pseudomonas* application, B_2= *Pseudomonas* application.

Table 5: Correlation coefficient of the traits

Traits	Numbers of grains / Panicle	1000 seeds weight (g)	Harvest index	Numbers of panicles / m^2	Grain yield kg ha^{-1}
Grain yield (kg ha^{-1})	1				
Numbers of panicles /m^2	0.518	1			
Harvest index	0.690^{**}	0.212^{ns}	1		
1000 seeds weight (g)	0.583^{**}	0.469^{**}	0.224^{ns}	1	
Numbers of grains per Panicle	0.318^{*}	0.241^{ns}	0.125^{ns}	0.131^{ns}	1

ns; nonsignificant; **, significant at $p \leq 0.01$; *, significant at $p \leq 0.05$.

the bacteria *Azospirillum lipoferum*. In comparison to the lowest yield production (2867 Kg ha^{-1} with the treatment of 25 kg of nitrogen without bacteria) a 39.7% increasing in yield was obtained (Table 2). *Azospirillum* inoculation could increase rice yield significantly by 1.6-10.5 g $plant^{-1}$ (32-81% increasing) in greenhouse conditions [32, 41]. However, in field conditions, the estimated yield increase was about 1800 kg ha^{-1} (22% increase) as reported by Balandreau [21]. Yield increment in rice due to inoculation of *Azospirillum* and *Azotobacter* has been reported in a range of 5-60% [21]. As shown in Table (3), rice grain yield significantly differed in various levels of nitrogen treatments and bacteria *Pseudomonas flouresence* at 5% of probability level. The highest rice grain yield was produced with the combination of 100 kg of nitrogen fertilizer and *Pseudomonas* bacteria (B_2), with an increasing of 56% compared to the lowest yield (25 kg ha^{-1} of nitrogen without the presence of *Pseudomonas* bacteria (B1). As it's know n, nitrogen has a major effect on plants growth and *Pseudomonas* bacteria as a growth promoting bacteria needs nitrogen as a nutrient, although they are a nitrogen fixer.

These bacteria increase nitrogen uptake efficiency and impacts rice yield. This study is consistent with Ramezanpour [42], who reported that bacteria *Pseudomonas* is capable of solving phosphorus which increases the yield. *Pseudomonas* bacteria also influence the ability of auxin production to increase rice yield up to 34%. The interactions between different levels of nitrogen and bacteria *Pseudomonas flouresence* and *Azospirillum lipoferum* on yield and yield components showed that the highest yield (5733 kg ha^{-1}) was produced with 100 kg of nitrogen intake with the bacteria *Pseudomonas flouresence* which compared to the lowest yield production from 25 kg of nitrogen treatment and no bacteria showed a 67% increment (Table 4). It must also be considered that 75 kg of nitrogen treatment with the bacteria *Pseudomonas flouresence* yielded 5467 kg ha^{-1} and showed no significant difference with the highest yield production. Considering the bacterial combined treatment (*Pseudomonas flouresence* and *Azospirillum lipoferum*) the highest yield production was by using 100 kg of nitrogen which compared to 25 kg of nitrogen, the yield increased 40% (Table 4). According to Fllik and Okon [43], when the population of the bacteria *Azospirillum* reaches a higher level of a certain amount, it prevents *Pseudomonas* activity in plants. Due to correlation coefficients (Table 5) 1000 seeds weight had a significant negative correlation with grain yield while panicle per square meter, grains per panicle and harvest index, all showed positive correlation (1%, 5% and 1%, respectively) with grain yield.

Number of Panicles per Square Meter: Appendix Table 5-1 and Tables 2, 3, 4 and 5 shows the results of analysis of variance and the means of the effects of the two bacteria and various nitrogen levels on number of panicles in square meter respectively. According to analysis of variance results, the nitrogen treatment significantly influenced panicles per square meter at $P<0.01$(Table 1).

The interaction between 100 kg nitrogen and bacteria *Pseudomonas flouresence* produced the highest number of panicles per m^2, which comparing to 25 kg nitrogen treatment shows a 28% increasement. Zabihi, *et al*. [44] showed that the inoculation of wheat seeds with the bacteria Pseudomonas increased 1000 seeds weight, number of clusters, number of grains per panicle and yield. The highest number of panicles with the mean of 84 produced with the combination between nitrogen and bacteria *Azospirillum lipoferum* was with 100 kg nitrogen treatment which had a 25% increment compared to 25 kg nitrogen (60.17) application (Table 2). The interaction of 100 kg nitrogen and bacteria *Pseudomonas flouresence* produced 84.67 panicles (Table 3). Although grains per panicle and harvest index had positive correlation with panicle per square meter but was not significant. Yield had a significant ($P<0.05$) positive correlation and 1000 seeds weight had a significant ($P<0.01$) negative correlation with numbers of panicle per square meter (Table 5).

Harvest Index: Table 1 and Tables 2, 3, 4 and 5 are the Analysis of variance and means comparison of different bacteria and amounts of nitrogen effects on harvest index respectively. According to the results the interaction between bacteria *Pseudomonas flouresence* and *Azospirillum lipoferum* with different levels of nitrogen showed significant difference at $P<0.01$ level of probability on harvest index (Table 1). Lucy *et al*. [45] reported that the benefits of plants inoculation with growth stimulants bacteria includes enhancement in number of indicators such as speed of germination, root growth, production per unit area, bio-control of pathogen agents, leaf area, chlorophyll content, resistance to drought, shoots and roots weight and microbial activity.

The highest rate of harvest index considering the interaction of bacteria *Azospirillum lipoferum* and various nitrogen levels obtained with 50 kg of nitrogen without bacteria inoculation following with 75 kg of nitrogen with bacteria inoculation. This suggests that bacteria

Azospirillum lipoferum does not have influence on harvest index (Table 2). The interaction of different levels of nitrogen and bacteria *Pseudomonas flouresence* showed that application of 50 and 75 kg nitrogen fertilizer with bacteria inoculation had the highest harvest index (48.52 and 47.93 respectively) both in group A (Table 3). As shown in the interaction of nitrogen and bacteria *Pseudomonas flouresence* and *Azospirillum lipoferum* mean comparison table, the highest harvest index obtained with the treatments of 25 and 50 kg nitrogen with the presence of *Pseudomonas flouresence* without *Azospirillum lipoferum* inoculation respectively (Table 4). The correlation coefficients table shows a 5% and 1% significant correlation between harvest index with yield and 1000 seeds weight respectively. Also yield and panicle per square meter (not significant) and number of grains per panicle (not significant) has positive and 1000 seeds weight has a negative correlation with harvest index (Table 5).

1000 seeds weight: Different amounts of bacteria and nitrogen levels had no significant effect on 1000 seeds weight (Table 1). The highest 1000 seeds weight among the composition of nitrogen and *Azospirillum lipoferum* obtained with the treatment of 100 kg of nitrogen without bacteria inoculation. Also, different treatments of nitrogen levels and bacteria inoculation did not show significant difference (Table 2). The interaction of nitrogen and both bacteria (*Pseudomonas flouresence* and *Azospirillum lipoferum*) showed that the highest 1000 seed weight was obtained with the application of 25 kg of nitrogen without the presence of both bacteria. As grain weight is an important indicator in wheat, this indicates that grain weight had been reduced by increasing the amount of nitrogen (Table 4). Research conducted on the effect of bacteria stimulating growth on this indicator suggests that some of these bacteria has a significant effect on growth parameters while some have no impact [46]. A significant ($P<0.01$) negative correlation is observed between 1000 seed weight and both panicle numbers and harvest index. Also grains per panicle and 1000 seed weight had no significant difference (Table 5).

Number of Grains per Panicle: Regarding Table 1, no significant difference had been observed among the treatments for number of grains per panicle. The highest number of grains per panicle regarding the interaction of different levels of nitrogen and bacteria *Azospirillum lipoferum* was observed in treatment with 75 kg of nitrogen and the presence of bacteria (Table 2). By considering the interaction of different levels of nitrogen and bacteria *Pseudomonas flouresence*, the highest number of grains per panicle were obtained with the application of 50 kg of nitrogen fertilizer without bacteria inoculation and 75 kg of nitrogen with bacteria inoculation (Table 3). The interaction between nitrogen and bacteria and on number of seeds clusters showed that the treatment of 50 kg of nitrogen with *Azospirillum lipoferum* and without *Pseudomonas flouresence* had the highest number of grains per panicle which in comparison to the treatment of 25 kg nitrogen without any bacteria inoculation showed 19% increment (Table 4). The number of grains per panicle and yield showed a significant difference ($P<0.01$). The number of grains per panicle had positive correlation with yield, number of panicle and harvest index and negative correlation with 1000 grains weight. Also number of panicle, harvest index and 1000 seed weight showed no significant differences with seed per panicle (Table 5).

CONCLUSION

Increment of agricultural performance over the past three decades has been achieved by degrading the environment and the emergence of problems such as soil erosion, pollution from chemical fertilizers and pesticides, water resources and have reduces biological diversity in plants and animals in the world. Therefore, low input farming systems as an aim to achieve maximum production in a short period of time is different to conventional system. Its aim is to achieve a stable level of production for long-term environmental compatibility to low energy inputs and small amounts of chemicals. Using these two types of bacteria in this study makes a better availability of nitrogen and phosphorus to plants that stimulate the issue of better growth, increase tolerance of plants against diseases and biotic and abiotic stress in order to stimulate further growth. The use of PGPR's increased the amount of cytokinin, gibberlin, auxin which led to better growth and increase rice yield. In fact, these bacteria by producing metabolites similar to growth regulatory substances directly increase plant growth and development. Based on the results of this study it can be stated that when phosphorus and nitrogen exist in soils the presence of PGPR's increase the absorption of elements in rice. Therefore the use of biological fertilizers results in yield increment decrease the use of chemical fertilizers so that the least adverse impact on the environment is achieved. This is recommended in sustainable agriculture. The results show that plants stimulating growth bacteria could improved growth characteristics including harvest index, panicle per square meter and seeds per panicle product which leads to yield increasement. Application of different amounts of nitrogen showed positive effects on increasing yield and yield components. Also the combination applications of bacteria and different amounts of nitrogen improved these characteristics. Tandon [47] showed that different stimulating growth bacteria have positive interaction on each other. Phosphorus efficiency will reach its highest level when it is applied with nitrogen and potash at the same time and its ratio with nitrogen should be 2:1 which has also been proved in this study. Therefore it is recommended that consumption of a balanced fertilizer with nitrogen and inoculated with appropriate bacteria could prevent the indiscriminate use of chemical fertilizers and reduce nitrate pollution which leads towards environmental survey.

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