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Influence of Nitrogen and Seed Biopriming with Plant Growth Promoting Rhizobacteria (PGPR) on Yield and Agronomic Characteristics of Red Lentil

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ABSTRACT

In order to study the effects of nitrogen fertilizer and seed inoculation with plant growth promoting rhizobacteria (PGPR) on yield, agronomic character sticks, rate and effective grain filling period of Red Lentil, a factorial experiment was conducted based on randomized complete block design with three replications in farm village in Ardabil in 2012. Factors were different rates of nitrogen fertilizer in four levels (without nitrogen application and application 25, 50 and 75 kg urea per hectare) as N_0, N_1, N_2, N_3 respectively and seed inoculation with plant growth promoting rhizobacteria in four levels (without inoculation, seed inoculation with *Azospirillum lipoferum* strain OF, *Azotobacter chrocococum* strain 5, *Pseudomonas* strain 9). The results showed that nitrogen fertilizer rates and seed inoculation with PGPR had significantly effects on grain yield, grian 1000 weight, number of grains per plant, plant height and all of grain filling parameters such as grainfilling period, rate and effective grain filling period. Maximum of grain filling period was obtained in application of 50 kg urea/ha × seed inoculation with *Azotobacter chrocococum*. Seed inoculation with plant growth promoting rhizobacteria increased grain yield and nitrogen use efficiency compared to no seed inoculation. Grain yield increased in high rates of nitrogen fertilizer and it was vice versa in fertilizer use efficiency. Maximum of nitrogen use efficiency (0.75 kg/kg) was obtained in application of 50 kg urea /ha × seed inoculation with *Azotobacter chrocococum* and minimum of it (0.22 kg/kg) was obtained in application of 75 kg urea /ha × no seed inoculation.
KEY WORDS: Nitrogen fertilizer, Plant Growth Promoting Rhizobacteria (PGPR), Red Lentil.

INTRODUCTION

Among the various problems that exist in human lives, always being fed and threatens him more comfortable. (Shirani Rad and Ahmadi, 1997). Unlike the other crops, toxic and antinutritional factors of lentils little has been reported. Requires a short time to cook lentils and beans are one of the most digestible, which may be used wholly or pulverized. The original product, a grain that has a relatively high proportion of protein, carbohydrates and calories than other cereals are (Muehlbauer et al, 1985). Lentil Dahl plant is highly resistant to drought and dry areas with less than 650 mm of rainfall per year, and even in tolerating the large number arid region that produces grain (malakouti and nafisi, 1992).

Active worlds first element nitrogen to plants that should be financed through the soil due to limited storage in the soil is the most important factor limiting plant growth (Chandrasekar et al, 2005).

Of nitrogen fertilizer because it is taking too much damage includes (Foulkes et al, 1998). Effective use of biological fertilizers to maintain soil quality management practices should be considered as desirable (Burelle et al, 2006).

Biological nitrogen fixation by legumes as an adjective is important. In this regard, the use of growth-promoting bacteria is one of the things that can improve plant growth and nutrition and environmental sanitation to be effective.

When the mineral nutrients in plants are lacking, symbiotic relationships can be beneficial to plant growth. Studies show that increasing the seed inoculated with bacteria growth mode of the inoculants increased. This increase was probably due to the presence of microbial populations in the rhizosphere soil by recycling nutrients and making it available, promote healthy root development during the competition origins of pathogens and increase the absorption of nutrients are plant growth (Roesti et al, 2006).

Materials and Methods**:**

The trial in 2012 on a farm in West Ardabil geographical coordinates 38 degrees and 15 minutes north latitude and 48 degrees 15 minutes West, and average annual rainfall was 380 mm during the growing season. Leaves red lentil varieties were used in this experiment. Factorial experiment in a randomized complete block design

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with three replications. The first factor involves bacterial nitrogen fixation in four levels (control or inoculated with bacteria, seed inoculation with bacteria Pseudomonas strains 9, Azotobacter chroococcum strains 5 and Azospirillum lipoferum strain OF) and the second consisting of N fertilizer at four levels (control or amounts of fertilizer and 25, 50 and 75 kg urea per ha). Seeds inoculated with bacteria for growth, with a suitable concentration of liquid water and gum Arabic than 10% by weight - volume produced.

Repeat for each piece of land was divided into 16 plots. Each plot consisted of four rows 25 cm apart and 2.5 m long. Between plants in the row of 4-3 cm were considered. Distance between main plots and blocks were taken from each 1 meter Fertilizers operating independently of each plot is adjacent plots. Planting and sowing seed on the line, at a depth of 2 cm and land preparation, including plowing, disk leveler that was done before planting. Nitrogen fertilizer use efficiency was calculated from the following equation:

$$\text{"Fertigation"} = \frac{\text{"Yield basin that received fertilizer"} - \text{"Yield basin that received no fertilizer"}}{\text{"The fertilizer consumption"}}$$

Immediately after planting the seeds and watering the next one irrigation during the growing season based on environmental conditions and plant needs to be done once every 12 days. Weed control during the growing season was done manually. To evaluate the effect of treatments on grain filling rate, after an initial period of flowering and grain filling, 8-stage sampling (every 5 days in duration) was performed.

In order to analyze and interpret the parameters related to the filling of a linear regression model (two-piece) using Proc NLIN procedure DUD and SAS software were used as follows.

$$GW = \begin{cases} a + bt, & t < t_0 \\ a + bt, & t \geq t_0 \end{cases} \qquad (2)$$

Where GW grain weight, t the time, b grain filling rate, t_0 is the period of grain filling and a y-intercept. Grain weight changes over time, this model is divided into two stages: First, the fact that the linear phase of grain filling, Seed weight to reach their maximum values at time t_0 is in fact the weighted maturity date, the linearly increases. At this point the slope of the regression line ($t < t_0$) represents the rate of grain filling. All the models fit the data with the two important parameters of grain filling rate, grain filling (b) and weighted maturity (t_0) the value t_0 then above was placed between the second and GW seed weight was estimated. To determine the effectiveness of the proposed filling of the relationship between Ellis and Pieta Filho (1992) was used as follows.

$$EFP = \frac{MGW}{GFR} \qquad (3)$$

In this regard, effective grain filling period EFP, MGW maximum grain weight and grain filling rate of GFR. Yield of the three central rows of each plot, considering the marginal effect of a square meter of the surface was measured. To measure the yield and other traits such as plant height, number of seeds per plant, seed weight and pod fill and empty, randomized trial of the main lines of 8 plants per unit of plant competition, and the randomly selected as the average value of the attribute was used to analyze the data. Data analysis using SAS software and the means obtained were compared using Duncan's multiple range test.

Filling:

The results of the analysis of variance table shows the effect of nitrogen fertilizer, seed inoculation with PGPR treatment combination effect of these two factors affect the rate and duration of grain filling was significant at the one percent level (Table 1). Longer than the period of transfer of assimilates from source to destination, thus providing increased grain yield (Grant, 1985). Effects of nitrogen fertilizer levels on grain filling rate and duration of seed inoculation with PGPR are given in Figures 1 to 4. The fitted equations for each treatment combination (Table 2) are presented.

Based on the results was determined by fitting the slope or rate of grain filling in seed inoculated with bacteria were similar (Table 3). These legumes are low in comparison with other legumes need nitrogen (Zafar et al, 2003). In conclusion, it seems that the use of urea fertilizer level of 50 kg ha nitrogen is sufficient so that the plant can increase the speed and rate of grain filling. The effective grain filling period, according to Table 2, it can be said that during this period at different levels of nitrogen fertilizer and PGPR were different, so that seed inoculation with the PGPR inoculation to increase the course in the case of (table 2).

Maximum duration of grain filling (35.51 days) in the 75 kg of urea per hectare and seed inoculation with Azotobacter and at least during this period (29.85 days) in the treatment combination of fertilizer and seed inoculation with bacteria was not. Slope of the fitted line in the usage of 75 kg urea per acre and seed inoculation with bacteria (0.00096), less the lack of fertilizer and seed inoculation with bacteria (0.00097), respectively (Table 2). Consequently, the treatment combination of 50 kg urea per hectare of seed inoculation with Azotobacter maximum grain filling rate and treatment combination of 75 kg of urea per hectare and seed inoculation with PGPR was the least of it. PGPR seems to produce growth hormone and nutrient supply may have provided increased grain filling rate (Daynard et al, 1971).

Yield and its components:

Plant height:

Analysis of variance (Table 3) showed that the effect of nitrogen and Plant height growth promoting bacteria and the effect of treatment combination of these two factors was significant at one per cent level of probability. Comparison of treated compounds showed the highest Plant height (49.5 cm) to the application of 50 kg urea per hectare of seed inoculation with Azotobacter and the lowest (29.5 cm) to the lack of fertilizer and seed inoculation with bacteria belonged to drive growth (table 5). The results Shehata and EL-Khawas (2003) were consistent. Plant height and rhizobacteria can increase productivity through Fitokrom synthesis, increased availability of food in one place, easy to absorb nutrients, reducing heavy metal toxicity in plants against pathogens and induce systemic resistance to pathogens increase (burd et al, 2000).

Number of seeds per plant:

Trait on seed yield in lentil is the most important and effective role in increasing grain Performance Is. Based on the analysis of variance (Table 3) under Tasyrmqdar nitrogen fertilizer plant, PGPR treatment combination effect of these two factors was significant at the one percent level. Mean comparison showed that the highest number of seeds per plant (511.38) in the 50 kg of urea per hectare and the lowest (450.23) in the case of fertilizer were estimated (Table 4). Seed inoculation with Azotobacter on the number of seeds per plant (551.40) and lowest (295.22) in non-inoculated respectively (Table 4).

Treatment combination of these two factors mean that the number of grains per 50 kg of urea per hectare of seed inoculation with Azotobacter (574.57) and the lowest (303.4) in the form of 75 kg of nitrogen fertilizer and seed inoculation with bacteria, respectively.

Components can increase the role of nitrogen-fixing bacteria growth and release it at critical stages that need to be linked to increased nitrogen fertilizer consumption at critical stages of growth. Brevendan et al, (1978) Thereby increasing the number of seeds per plant increased performance, increase the number of nodes per shoot and flowers and pod loss reported.

Seed weight:

Based on the analysis of variance table (Table 3) affected by nitrogen fertilizer seed weight, and PGPR treatment combination of these two factors was significant at the one percent level. Mean comparison showed that the highest seed weight (52.29 g) consumed 50 kg of urea per hectare and the lowest (44.98 g) in the case of fertilizer respectively. The highest yield of seed inoculation with Azotobacter (54.60 g) and the lowest (36.22 g) in the case of non-inoculated respectively (Table 4). The mean effect of treatment combination levels of nitrogen fertilizer and seed inoculation with bacterial growth showed that the highest seed weight (57.244 mg) in eat 50 kg of urea per hectare in the case of seed inoculation with Azotobacter and lowest (33.36 g) in case of non-inoculated and fertilizer were obtained (table 5). Idris research (2003) showed that inoculation with bacteria Azotobacter seed weight is increased.

Number of pods per plant:

Based on the analysis of variance (Table 3), number of pods per plant affected by nitrogen fertilizer and PGPR was significant at the one percent level. But the effect of experimental treatments on these traits were not significant. Mean comparison showed that the highest number of pods per plant (32.92) with 50 kg of urea per hectare and the lowest (23.40) in the case of fertilizer respectively. Seed inoculation with Azotobacter on the number of seeds per plant (30.13) and lowest (24.99) in non-inoculated respectively (Table 4). Togay and colleagues (2008) reported that the lentil yield per unit area is influenced by the number of pods and seeds per plant and number of pods and seeds of higher plants, thereby increasing the yield per unit area.

Seed yield per unit area:

Based on the analysis of variance table (Table 3) red lentil Seed yield is affected by nitrogen fertilizer, and PGPR treatment combination of these two factors was significant at the one percent level. Mean comparison showed that the highest Seed yield (2748.78 kg ha) consumed 50 kg of urea per hectare and the lowest (2153.18 kg per ha) was estimated in the absence of fertilizer. The highest Seed yield of seed inoculation with Azotobacter (2885.40 kg per ha) and lowest in the non-inoculated (2038.16 kg per ha) was calculated (Table 4). The mean effect of treatment combination of these two factors showed that the highest Seed yield (3182.92 kg per hectare) of 50 kg urea per hectare of seed inoculation with Azotobacter and lowest (1689.47 kg per hectare) in the case of non-inoculated and non-fertilizer, respectively (table 5). It looks at the amount of 50 kg of fertilizer per hectare, using appropriate amounts of nitrogen fertilizer has caused red lentils with root system is better able to absorb more nitrogen to increase yield.

Nitrogen use efficiency:

According to the analysis of variance table (Table 6) Nitrogen use efficiency was influenced by the experimental treatments. The performance of seed inoculation with PGPR significantly increased compared to non-inoculated with bacteria. The growth of bacteria, Azotobacter accounted for the highest efficiency (Table 7).

Combined effect of seed inoculation with bacterial growth at different levels of nitrogen fertilizer on nitrogen use efficiency was significant at the five percent level (Table 6). The highest nitrogen use efficiency (20.57 kg/kg) consumed 25 kg of urea per hectare in the case of inoculation with Azotobacter and lowest (4.01 kg/kg) fertilizer at 75 kg per hectare in the case of inoculation with bacteria belonging (table 8).

The final conclusions**:**

Different levels of nitrogen fertilizer on grain filling process and the application of PGPR showed that the pattern of seed development at all levels of fertilizer and PGPR similar. Thus, different types of bacteria to the seed weight increased linearly and reached its maximum. After this stage, seed weight was not much change in the form of a horizontal line. The results revealed that the bacteria growth at different levels of nitrogen fertilizer, the effective filling period, maximum grain weight and grain filling course there are differences. In other words, the maximum grain filling rate, grain filling period and a maximum weight of 50 kg urea per hectare in the case of treatment combination of seed inoculation with Azotobacter respectively.

Nitrogen fertilizer with seed inoculation with PGPR increased grain yield and most of the traits. Between different levels of fertilizer consumption of 50 kg and the growth of bacteria, Azotobacter inoculated seed yield was accounted for. It seems appropriate to apply a combination of bacteria and nitrogen fertilizer use red lentils have a positive impact on performance and Continuous use of chemical fertilizers can be used instead of the optimal inputs towards sustainable agriculture and reducing environmental pollution from chemical fertilizers step.

Figure 1 - The trend of grain filling rate of nitrogen fertilizer

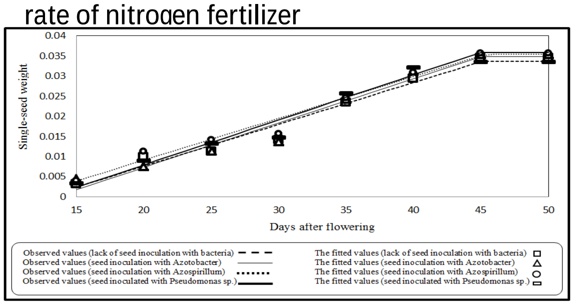

Figure 2 - Trends in grain filling rate of 25 kg nitrogen fertilizer

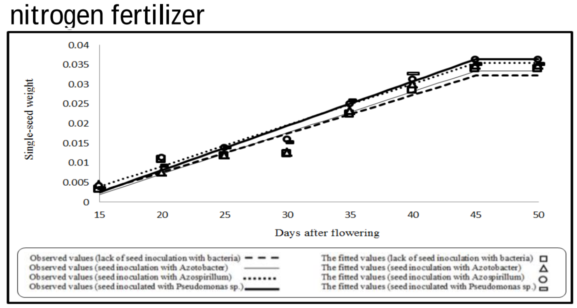

Figure 3 - Trends in grain filling rate of 50 kg nitrogen fertilizer

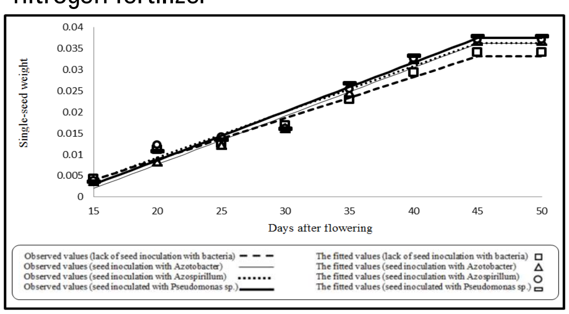

Figure 4 - Trends in grain filling rate of 75 kg nitrogen fertilizer

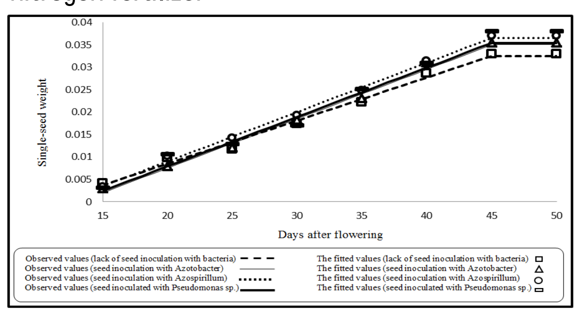

Table 1 - Analysis of variance of seed inoculation with PGPR and nitrogen fertilizer effects on grain filling rate and duration of red lentils

		Mean square		
Sources of change	**df**	**Grain filling Period**	**Effective grain filling period**	**Grain filling rate**
Repeat	2	1321.358850**	712.216017**	0.00000385*
Nitrogen fertilizer	3	2.208369**	2.110843**	0.00000155ns
PGPR	3	2.460319**	18.998683**	0.00000517*
Bacteria* Nitrogen	9	2.830985**	4.414042**	0.00000409ns
Error	30	1.0522931	2.642002	0.00013624
The coefficient of variation(percent)	-	4.12305	8.89934	13.30316

ns, * and **, respectively, non-significant and significant at the five and one percent level.

Table 2 - Effect of different levels of nitrogen fertilizer and PGPR effect on the rate and duration of grain filling and the slope of the fitted line

Treatment combination	Grain filling period	Effective grain filling period (days)	Grain filling rate (mg per day)	Fitted equation
Lack of nitrogen fertilizer * Non-inoculated	42.770 j	29.850 h	0.00097 hg	y= -0.0105+0.00097x
Lack of nitrogen fertilizer * Inoculated with Bacteria Pseudomonas	45.640 feg	32.955 de	0.00112 c	y= -0.0145+0.00112x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azospirillum	45.730 fed	33.268 dc	0.00104 f	y= -0.0117+0.00104x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azotobacter	46.000 cbd	34.326 b	0.0011 d	y= -0.0147+0.0011x
Lack of nitrogen fertilizer * Non-inoculated	43.850 i	31.150 g	0.00098 g	y= -0.012+0.00098x
Lack of nitrogen fertilizer * Inoculated with Bacteria Pseudomonas	45.850 ced	33.141 dc	0.00113 bc	y= -0.0144+0.00113x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azospirillum	45.400 hg	34.270 b	0.00105 f	y= -0.0118+0.00105x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azotobacter	46.120 cb	34.571 b	0.00105 f	y= -0.0138+0.00105x
Lack of nitrogen fertilizer * Non-inoculated	45.330 hg	32.180 f	0.00104 f	y= -0.0132+0.00104x
Lack of nitrogen fertilizer * Inoculated with Bacteria Pseudomonas	45.450 fhg	32.542 fe	0.00114 ab	y= -0.0151+0.00114x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azospirillum	45.600 feg	34.351 b	0.001.8 e	y= -0.0123+0.00108x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azotobacter	45.780 ed	35.154 a	0.00115 a	y= -0.0143+0.00115x
Lack of nitrogen fertilizer * Non-inoculated	45.170 h	32.180 f	0.00096 h	y= -0.0107+0.00096x
Lack of nitrogen fertilizer * Inoculated with Bacteria Pseudomonas	45.370 hg	33.545 c	0.0011 d	y= -0.0141+0.0011x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azospirillum	46.300 b	34.545 b	0.0011 d	y= -0.0130+0.0011x
Lack of nitrogen fertilizer * Inoculated with Bacteria Azotobacter	46.650 a	35.510 a	0.0011 d	y= -0.0145+0.0011x
LSD	0.312	0.528	0.00002	

Out with the same letters in each column with no significant difference.

Table 3- Analysis of variance of red lentils in different amounts of nitrogen fertilizer and seed inoculation with bacteria PGPR.

		Mean-square				
Sources of change	**Degrees of freedom**	**Plant height**	**Number of seeds per plant**	**Thousand seed weight**	**Number of pods per plant**	**Performance Is**
Repeat	2	43.93 **	99.13 ns	4.29 ns	10.81 ns	7799.07 ns
Nitrogen	3	462.07 **	31295.04 **	448.89 **	549.49 **	849214.48 **
Inoculum levels	3	186.71 **	499930.85 **	2548.44 **	191.29 **	1545562.51 **
Insemination * Nitrogen	9	25.83 **	17324.51 **	233.75 **	77.42 ns	81319.36 **
Experimental error	30	108.85	17879.57	96.47	177.50	9264.02
The coefficient of variation (percent)	—	4.95	5.22	3.74	8.58	3.81

ns, * and **, respectively, non-significant and significant at the five and one percent level.

Table 4 - Comparison of the average effect of nitrogen fertilizer rates on yield and some Wu bacteria growth traits in red lentils.

		Plant height (cm)	Number of seeds per plant	Thousand seed weight (gr)	Number of pods per plant	Performance Is (kg/ha)
Levels of urea nitrogen consumption (kilograms per hectare)	Zero	32.16 d	450.88 b	45.09 c	23.40 c	2153.18 c
	25	34.95 c	450.23 b	44.98 c	28.07 b	2521.23 b
	50	46.22 a	511.38 a	52.29 a	32.92 a	2748.78 a
	75	40.28 b	457.08 b	49.22 b	28.92 b	2679.81 a
Different levels of seed inoculation	Non-inoculated	33.14 c	295.22 c	36.22 c	24.99 b	2038.16 d
	Inoculated with Pseudomonas	41.02 a	488.33 b	47.31 b	28.68 a	2515.15 c
	Inoculated with Azospirillum	37.64 b	534.63 a	53.43 a	29.51 a	2664.28 b
	Inoculated with Azotobacter	41.80 a	551.40 a	54.60 a	30.13 a	2885.40 a

Out with the same letters in each column with no significant difference.

Table 5 - Comparison of the effects of different levels of nitrogen fertilizer treatment combination of seed inoculation with PGPR on some characteristics of red lentils.

The amount of fertilizer	Bacteria	Plant height (cm)	Number of seeds per plant	Thousand seed weight (gr)	Performance Is (kg / ha)
Lack of fertilizer	Non-inoculated	29.580 [f]	303.40 [g]	33.367 [f]	1689.47 [j]
	Pseudomonas	32.368 [ef]	442.35 [e]	39.503 [e]	2072.50 [h]
	Azospirillum	30.563 [f]	514.18 [dc]	53.398 [b]	2336.25 [g]
	Azotobacter	36.145 [d]	543.62 [bdac]	54.097 [b]	2514.50 [f]
Use 25 (kg / he) nitrogen fertilizer	Non-inoculated	30.084 [f]	277.77 [hg]	34.919 [f]	1878.52 [i]
	Pseudomonas	36.577 [d]	448.91 [e]	43.053 [d]	2563.33 [f]
	Azospirillum	36.492 [d]	530.42 [bdc]	48.767 [c]	2614.22 [fe]
	Azotobacter	36.648 [d]	543.84 [bdac]	53.198 [b]	3028.83 [ba]
Use 50 (kg / he) nitrogen fertilizer	Non-inoculated	37.307 [d]	351.18 [f]	40.356 [ed]	2089.77 [h]
	Pseudomonas	49.421 [a]	553.29 [bac]	54.430 [ba]	2774.50 [de]
	Azospirillum	48.585 [ba]	566.49 [ba]	57.132 [a]	2947.92 [bc]
	Azotobacter	49.572 [a]	574.57 [a]	57.244 [a]	3182.92 [a]
Use 75 (kg / he) nitrogen fertilizer	Non-inoculated	35.605 [d]	248.54 [h]	36.251 [f]	2494.90 [fg]
	Pseudomonas	45.741 [bc]	508.79 [d]	52.274 [b]	2650.25 [fe]
	Azospirillum	34.950 [ed]	527.45 [bdc]	54.457 [ba]	2758.75 [de]
	Azotobacter	44.852 [c]	543.58 [bdac]	53.897 [b]	2815.33 [dc]

Out with the same letters in each column with no significant difference.

Table 6 - Analysis of variance of different levels of nitrogen fertilizer and seed inoculation with PGPR on nitrogen use efficiency of red lentils

Sources of change	Degrees of freedom	Mean square
Repeat	2	0.56 [ns]
Nitrogen use	2	182.20 **
Bacterial growth	3	51.29 *
Bacteria * Nitrogen	6	59.29 **
Error	22	13.04
The coefficient of variation (percent)	–	5.16

ns, * and **, respectively, non-significant and significant at the five and one percent level.

Table 7 - Comparison of mean levels of nitrogen fertilizer and bacteria growth on nitrogen use efficiency of red lentils

Treatment factors examined		Nitrogen use efficiency (kg kg)
The levels of urea nitrogen fertilizer (kg ha)	25	14.72 [a]
	50	11.91 [a]
	75	7.02 [b]
Seed inoculation with PGPR	Non-inoculated	8.76 [c]
	Pseudomonas	13.79 [a]
	Azospirillum	9.66 [bc]
	Azotobacter	12.65 [ba]

Non-shared letters in each column mean differences are statistically significant.

Table 8 - Comparison of different levels of nitrogen fertilizer treatment combination of seed inoculation on nitrogen use efficiency with PGPR

Fertilizer use	Bacteria	Nitrogen use efficiency (kg / kg)
Use 25 (kg / he) nitrogen fertilizer	Non-inoculated	7.562 [dfe]
	Pseudomonas	19.633 [ba]
	Azospirillum	11.119 [dce]
	Azotobacter	20.573 [a]
Use 50 (kg / he) nitrogen fertilizer	Non-inoculated	8.006 [dfce]
	Pseudomonas	14.040 [bc]
	Azospirillum	12.233 [dc]
	Azotobacter	13.368 [dc]
Use 75 (kg / he) nitrogen fertilizer	Non-inoculated	4.011 [dc]
	Pseudomonas	7.703 [e]
	Azospirillum	5.633 [fe]
	Azotobacter	10.739 [dce]

Non-shared letters in each column mean differences are statistically significant.

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