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Assess Effect of Nitrogen Fertilizer and Phosphorous Biofertilizers (Azospirillum and Pseudomonas) on Barley Crop Production and Seed Protein Content

OPEN ACCESS

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Azospirillum is effective in biostabilization of nitrogen and Pseudomonas is effective in dissolving soil phosphorus.

OBJECTIVES: Current study was conducted to assess effect of different level of nitrogen and biofertilizer on seed yield, its components and protein content.

METHODS: This research was carried out via spilt plot factorial experiment based on randomized complete blocks design with three replications along 2014-2015 year. The main factor included nitrogen ($N_{0\%}=0$ or control, $N_{50\%}=75$, $N_{75\%}=112.5$, $N_{100\%}=150$ kg.ha⁻¹). The sub factor at first consisted Azospirillum (Azo₀: non use of Azospirillum or control, Azo₁: use of Azospirillum) and then Pseudomonas (Pse₀: non use of Pseudomonas or control, Pse₁: use of Pseudomonas).

RESULT: The results showed that the application of nitrogen fertilizer had a significant effect on seed protein content, seed protein yield, plant height and seed yield at 1% probability level, also inoculation of Azospirillum increased the effect of nitrogen fertilizer on these traits. Mean comparison result of different level of Pseudomonas indicated that maximum seed yield (4800 kg.ha⁻¹) was noted for Pse1 and minimum of that belonged to control treatment. So Pseudomonas inoculation increased seed yield by 8% compared to the control treatment (no bacterial inoculation). As for Duncan classification made with respect to interaction effect N × Azo the maximum and minimum amount of seed yield belonged to N_{100%} and Azo₁ (5710.9 kg.ha⁻¹) and control (3981.3 kg.ha⁻¹). Inoculation of the plant with Azospirillum increased the seed protein content by 14.8%. Also inoculation of Pseudomonas caused the highest amount of seed protein content of 13.56 (g.100 g⁻¹).

CONCLUSION: Inoculation of Pseudomonas fluorescence also had a significant effect on all the mentioned traits. Inoculation of Azospirillum and 75 kg N ha⁻¹ significantly increased seed yield and the use of Azospirillum led to save 25% of the nitrogen fertilizer and can be advised to producers.

KEYWORDS: Cereal, Nutrition, Quantitative trait, Urea, Yield.

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1. BACKGROUND

Applying ecological principles and concepts, including the management and use of soil microorganisms and the relationships between them, can help us produce food more sustainably. Many researchers believe that one of the basic pillars of sustainable agriculture is the use of biofertilizers in agricultural ecosystems with the aim of eliminating or reducing the use of chemical inputs. The researchers said that the use of growth-promoting bacteria, while reducing the use and increasing the efficiency of chemical fertilizers, increased plant growth by increasing the uptake of nitrogen and phosphorus (Arruda et al., 2013). The use of chemical fertilizers as the fastest way to compensate for soil nutrient deficiencies and high yields has expanded dramatically but in many cases, the use of these fertilizers causes environmental pollution and ecological damage and increases production costs (Salehi et al., 2014). Excessive use of chemical fertilizers destroys the physical and chemical properties of the soil (Kalhapure et al., 2013). Among the elements, nitrogen affects the yield of the product more than other nutrients (Padilla et al., 2015). Excessive use of nitrogen fertilizers leads to nitrogen loss through leaching, groundwater pollution, sublimation of this element (air pollution), increasing the rate of decomposition of organic matter in the soil. Thus, large amounts of nitrogen oxide (NO₂) and carbon dioxide enter the atmosphere and these greenhouse gases are effective in warming the climate (Zaidi et al., 2017). Among the nitrogen-fixing bacteria are Azospirillum (Bashan and Holguin, 1997). Bacteria of the genus Azospirillum and Pseudomonas are the most important bacteria that stimulate plant growth. Also Azospirillum, to bio-stabilizing nitrogen and Pseudomonas, in addition

to dissolving soil phosphorus, affects the growth and yield of crops by producing significant amounts of growthstimulating hormones, especially auxin, gibberellin and the cytokine (Sharma, 2003). Phosphate-solubilizing microorganisms release phosphorus from insoluble sources and increase plant growth and yield. Jarak et al. (2012) showed that combined inoculation of corn with Pseudomonas fluorescens, Bacillus and Azotobacter crococum significantly increased plant yield. Askary et al. (2009) reported these bacteria increase number of lateral and capillary roots, which increases root area and increases access to water and nutrients, thus improving water status of the plant.

2. OBJECTIVES

Current study was conducted to assess effect of different level of nitrogen and biofertilizer on barley crop production and protein content.

3. MATERIALS AND METHODS

3.1. Field and Treatment Information

This research was carried out to evaluate effect of nitrogen and biofertilizer on quantitative and qualitative traits of barley via spilt plot factorial experiment based on randomized complete blocks design with three replications along 2014-2015 year. Place of research was located in Karaj city at longitude 51°56'E and latitude 35°45'N in Alborz province (North of Iran). The main factor included nitrogen (N_{0%}=0 or control, N_{50%}=75 kg.ha⁻¹, N_{75%}=112.5 kg.ha⁻¹, $N_{100\%}=150$ kg.ha⁻¹). The sub factor at first consisted Azospirillum (Azo₀: non use of Azospirillum or control, Azo₁: use of Azospirillum) and then Pseudomonas (Pse₀: non use of Pseudomonas or control, Pse₁: use of Pseudomonas). Each plot consisted of 6 lines with a distance of 50 cm and 5 meters length.

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3.2. Farm Management

The bacteria used in this study were amplified in the biology department of Tehran Soil and Water Research Institute. The bacterial population was 10⁸ bacterial cells per gram in inoculum matter. Based on the physical and chemical properties of the farm soil (Table 1) at a rate of 100 (kg.ha⁻¹) triple superphosphate was evenly distributed throughout the land. The Nitrogen fertilizer from urea source was distributed based on the treatments in the desired plots, 50% of which was distributed at the time of planting in early November and the remaining 50% before flowering consumed. Before the planting, a deep plow and two discs perpendicular to each other were done. The barley cultivar used in this experiment was Reyhan cultivar, which are a high yielding, early ripening and semi sensitive to cold stress. The seeds were weighed equally for all the treatments and after inoculated the seeds with the bacteria in different treatments were hand distributed on the surface of each plot. The amount of seeds was used considered based on 300 plants per square meter.

Table 1. Physical and chemical results of soil testing site

Depth (cm)	EC (ds.m ⁻¹)	рН	N (%)	P (ppm)	K (ppm)	Sand (%)	Clay (%)	Silt (%)
0-30	1.10	7.8	0.09	10	350	32	22.7	45.7
30-60	1.85	6.5-7.5	0.25	10-15	300-350	30.2	18.1	50.9

3.3. Measured Traits

To measure plant height, five plants were randomly selected and calculated as the desired height with using a ruler and a vector data meter. In order to measure seed protein, the amount of seed nitrogen content was first measured by Kjeldahl method. Then the percentage of seed protein content was calculated by multiplying the amount of nitrogen to the specific protein factor for barley (5.7) (Johnson and Wilrinson, 1992). From each experimental plot, 10 plants were harvested taking to determine the final yield. The area occupied by these 10 plants was calculated as the final yield was estimated in terms of square meters. Yield components in a crop are components of the final production of the plant and each crop has its own components. Yield components in barley plant include number of spike per plant, number of seeds per spike and 1000-seed weight, which were harvested and measured in 10 plants.

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS software (Ver.8) and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. Seed yield

According result of analysis of variance effect of nitrogen (N), Azospirillum (Azo), Pseudomonas (Pse) and interaction effect of $N \times Azo$ on seed yield was significant at 5% probability level but interaction effect of N \times Pse, Azo \times Pse and N \times Azo \times Pse treatments was not significant (Table 2). Mean comparison result of different level of Pseudomonas indicated that maximum seed yield (4800 kg.ha⁻¹) was noted for Pse₁ and minimum of that belonged to control treatment (Fig.1). So Pseudomonas inoculation increased seed yield by 8% compared to the control treatment (no bacterial inoculation).

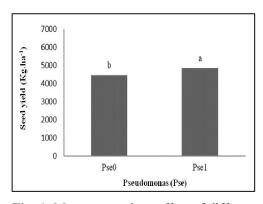


Fig. 1. Mean comparison effect of different level of Pseudomonas (Pse) on seed yield via Duncan test at 5% probability level.

As for Duncan classification made with respect to interaction effect N × Azo the maximum and minimum amount of seed yield belonged to N100% and Azo1 (5710.9 kg.ha⁻¹) and control (3981.3 kg.ha⁻¹) (Fig.2). It seems that due to the positive role of bacteria in the regulation and production of the growthpromoting hormones along with nitrogen fertilizer, it leads to better root development and causes more absorption of water and nutrients and increases yield. Nitrogen increases the durability of the leaf surface, especially during the flowering stage, and therefore increases the photosynthetic material during the growing season and increases the number of flowering branches. Increasing the number of flowering branches increases the number of seeds per plant and ultimately increases seed yield. Nitrogen increases the rate of cell division in plant growth due to its role in the production and release of the hormone cytokinin from the roots to the shoots. In addition, nitrogen indirectly affects the hormone gibberellin through cytokinin, thus increasing the growth of flowering branches and leaves and photosynthesis, thus ultimately causing the highest yield (Masoumi, 2011).

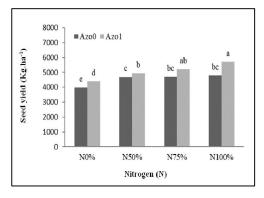


Fig. 2. Mean comparison interaction effect of different level of nitrogen (N) and Azospirillum (Azo) and on seed yield via Duncan test at 5% probability level.

S.O.V	df	Seed yield	No. seed per spike	No. spike per m ⁻²	Seed protein content	Seed protein yield	Harvest index
Replication	2	156181	50.769	29039.8	3.48 ^{ns}	194477	16.699
Nitrogen (N)	3	398120*	16.595 ^{ns}	19977.38*	40.24*	459164**	14.23 ^{ns}
Error I	6	345849	32.23	311278	3.15	223790	31.58 ^{ns}
Azospirillum (Azo)	1	714563*	75.75*	20833.3*	37.84*	663453**	1.22 ^{ns}
Pseudomonas (Pse)	1	151727*	23.10*	169456.3*	24.48*	296521**	45.14 ^{ns}
N × Azo	3	124206*	16.85 ^{ns}	684.27 ^{ns}	0.5077^{ns}	435582**	37.37 ^{ns}
N × Pse	3	276497 ^{ns}	2.84 ^{ns}	835.94 ^{ns}	3.66 ^{ns}	106829 ^{ns}	22.00 ^{ns}
Azo × Pse	1	330008^{ns}	5.4 ^{ns}	28834847*	0.4218 ^{ns}	40093911**	26.08 ^{ns}
N × Azo × Pse	3	338089 ^{ns}	11.37 ^{ns}	4787.7 ^{ns}	3.288 ^{ns}	197863 ^{ns}	17.52 ^{ns}
Error II	24	2064	8.101	3266	1.643	1014	26.20 ^{ns}
CV (%)	-	6.25	6.17	9.24	9.98	10.72	12.56

Table 2. Analysis of variance of measured traits

*,** and ^{ns} are significant at 1%, 5%, and non significant levels, respectively.

4.2. Number of seed per spike

Result of analysis of variance revealed effect of Azospirillum (Azo) and Pseudomonas (Pse) on number of seed per spike was significant at 5% probability level but effect of nitrogen (N), interaction effect of N \times Azo, N \times Pse, Azo \times Pse and N \times Azo \times Pse treatments was not significant (Table 2). According result of mean comparison of Pseudomonas maximum of number of seed per spike (42) was obtained for Pse₁ and minimum of that (40) was for control treatment (Fig.3). Evaluation mean comparison result indicated in different level of Azospirillum the maximum number of seed per spike (43.5) was noted for Azo₁ and minimum of that (40.5) belonged to control treatment (Fig.4). So application of the Azospirillum and Pseudomonas increased the number of seeds per spike by 6% and 4.9%, respectively. The presence of Pseudomonas bacteria is evident in these results due to the role that phosphorus plays in reproductive growth and seed formation in plants. The increase in vield due to inoculation with Azospirillum can be considered as the result of increasing the number of seeds per spike. An increase in the number of seeds per spike due to inoculation with Pseudomonas in barley has also been reported by Mehrvarz et al. (2008). Also Öztürk et al. (2003) reported an increase in the number of seeds per spike in barley by inoculate with Azospirillum.

4.3. Number of spike per square meter

According result of ANOVA effect of nitrogen (N), Azospirillum (Azo), Pseudomonas (Pse) and interaction effect of Azo \times Pse on number of spike per square meter was significant at 5% probability level but interaction effect N \times Azo, N \times Pse and N \times Azo \times Pse treatments was not significant (Table 2).

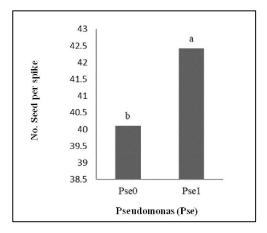


Fig. 3. Mean comparison effect of different level of Pseudomonas (Pse) on number of seed per spike via Duncan test at 5% probability level.

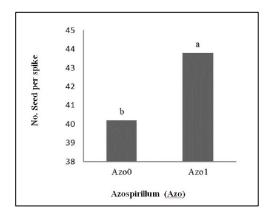


Fig. 4. Mean comparison effect of different level of Azospirillum (Azo) on number of seed per spike via Duncan test at 5% probability level.

Assessment mean comparison result indicated in different level of nitrogen the maximum number of spike per square meter (569) was noted for N_{100%} and minimum of that (481) belonged to N_{0%} (Fig.5). So the N_{100%} treatment had an increase of 18.1% compared to the control treatment. The results of Ilyas and Baho (2010) showed that the number of fertile tillers and the number of spikes per square meter increased significantly by inoculating Azospirillum with wheat seeds.

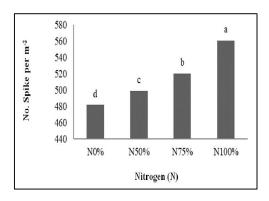


Fig. 5. Mean comparison effect of different level of Nitrogen (N) on number of spike per m^{-2} via Duncan test at 5% probability level.

The mean comparison of interaction effect of Azo \times Pse revealed, Azo₁ \times Pse₁ treatment with the production of 576 spikes per square meter and 30.3% increase compared to the control has the highest number of spikes per square meter. And $Azo_0 \times Pse_0$ treatment with the production of 442 spikes per square meter has the lowest number of spikes per square meter (Fig.6). Simultaneous application $Azo_1 \times Pse_1$ Produced the highest number of spikes per square meter so by compared to Azo₀Pse₀, Azo₁Pse₀ and Azo₀Pse₁ treatments, has an increase of 30.3%, 4% and 17.5%, respectively, is observed. Which indicates the greater impact of the simultaneous effect of use Azo1Pse1 on the number of spikes per square meter. Which shows the synergistic effects between Azo and Pse. Based on the mentioned results, it seems that the combination of different types of growthpromoting bacteria can provide the possibility of establishing a synergistic and intensifying relationship on growth and development and antagonistic effect for growth-reducing factors. So the result is an increase in the beneficial effects of bacteria in increasing plant growth and development and ultimately more crop production in the plant.

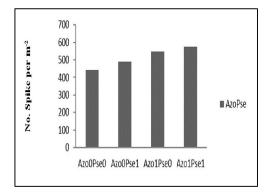


Fig. 6. Mean comparison interaction effect of Azospirillum (Azo) and Pseudomonas (Pse) on number of spike per m^{-2} via Duncan test at 5% probability level.

4.4. Seed protein content

Result of analysis of variance revealed effect of nitrogen (N), Azospirillum (Azo), Pseudomonas (Pse) on seed protein content was significant at 5% probability level but effect of nitrogen (N), interaction effect of N \times Azo, N \times Pse, Azo \times Pse and N \times Azo \times Pse treatments was not significant (Table 2). The results of mean comparison showed that inoculation of the plant with Azospirillum increased the seed protein content by 14.8% (Fig.7). It seems that inoculating wheat, barley and oats with Azospirillum due to increased nitrogen storage in the whole plant leads to an increase in seed protein content. As can be seen in Fig. 8, inoculation of Pseudomonas caused the highest amount of seed protein content of 13.56 (g.100 g^{-1}) and non-inoculation caused the lowest amount of seed protein content of 12.13 $(g.100 g^{-1})$ in barley. It seems that inoculation with Azospirillum and Pseudomonas has been effective on the parameters of the root system through hormonal mechanisms and led to increase the depth of the established roots and more absorption of nutrients such as nitrogen. Pseudomonas increase the phosphorus uptake by dissolving insoluble phosphate and increasing the amount of available phosphorus, resulting in nitrogen uptake (Raei Pour and Asgharzad, 2007). Based on the results (Fig. 9), the application of nitrogen was able to increase grain protein. N_{100%} treatment had the highest amount of seed protein content with an increase of 36.5% compared to the control treatment. In an experiment on barley and wheat at different levels of nitrogen (0, 40 and 80 kg.ha⁻¹), they reported a significant increase in the percentage of seed protein content (Öztürk et al., 2003). Also reported Azotobacter inoculation increased percentage of seed protein content of wheat with use nitrogen fertilizer (Egamberdiyeva and Hoflich, 2003). In a similar study, inoculation of wheat seeds with plant growthpromoting bacteria and simultaneous application of nitrogen fertilizer had a significant effect on the seed nitrogen content of wheat and straw.

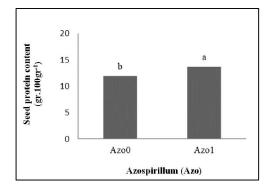


Fig. 7. Mean comparison effect of the Azospirillum (Azo) on seed protein content via Duncan test at 5% probability level.

4.5. Seed protein yield

According result of ANOVA effect of nitrogen (N), Azospirillum (Azo), Pseudomonas (Pse) and interaction effect of N \times Azo and Azo \times Pse on seed protein yield was significant at 1% probability level but interaction effect N \times Pse and N \times Azo \times Pse was not significant (Table 2).

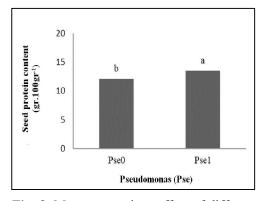


Fig. 8. Mean comparison effect of different level of Pseudomonas (Pse) on seed protein content via Duncan test at 5% probability level.

The results of comparing the mean interaction of the Azospirillum and Pseudomonas showed that it produced the highest seed protein yield which indicates the aggravating and synergistic effects between the Azospirillum and Pseudomonas (Fig. 10). Given that Azospirillum is one of the nitrogen fixing bacteria and this nutrient is the raw material of protein formation, probably one of the reasons for increasing the amount of protein by inoculating Azospirillum bacteria is nitrogen fixation by this bacterium. This result can indicate the strengthening relationship of the combination of the mentioned bacteria with each other in order to increase root growth and nutrient uptake by wheat plants (Zahir et al., 2004). In the production and regulation of growth-promoting hormones by expanding the roots and absorbing water and nutrients in nitrogen uptake improves growth and increases photosynthesis and production of assimilate and since a lot of energy is needed to stabilize nitrogen, it is supplied with sufficient phosphorus and abundant ATP, which leads to increased grain yield and grain protein yield (Egamberdiyeva and Hoflich, 2003).

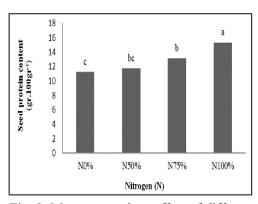


Fig. 9. Mean comparison effect of different level of Nitrogen (N) on seed protein content via Duncan test at 5% probability level.

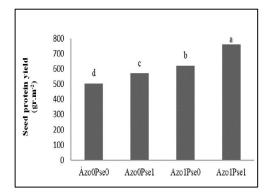


Fig. 10. Mean comparison interaction effect of Azospirillum (Azo) and Pseudomonas (Pse) on seed protein yield via Duncan test at 5% probability level.

As shown in Fig. 11, the greatest effect of Azospirillum is observed at the N100% \times Azo₁ treatment, which indicates a greater additive effect of Azospirillum in this treatment than other treatments. It seems that bacterial coexistence has improved nitrogen concentration and increased seed yield and finally seed protein yield by improving growth and development and subsequent increase in plant dry weight along with nitrogen fertilizer. Agni et al. (2012) reported the highest amount of protein from the combined treatment of biological fertilizer with half of chemical fertilizer. They stated that the activity of nitrogen-fixing bacteria increased the recycling rate of nitrogen fertilizer

by providing part of the required nitrogen during the growing season and reducing its losses. In this regard, the estimate of the overall efficiency of fertilizer application is about 50% or less than 50% for nitrogen, about 10% for phosphorus was reported (MalekiNarg *et al.*, 2013). Öztürk *et al.* (2003) they also obtained similar results regarding the positive effects of Azospirillum and nitrogen.

4.6. Harvest index

Result of ANOVA revealed effect of nitrogen (N), Azospirillum (Azo), Pseudomonas (Pse), N \times Azo, N \times Pse, Azo \times Pse and N \times Azo \times Pse on harvest index was not significant (Table 2).

5. CONCLUSION

The results of this experiment showed that inoculation of the plant growth-promoting bacteria had a positive and increasing effect on most of the traits measured in this experiment, either alone or simultaneously with the nitrogen fertilizer application.

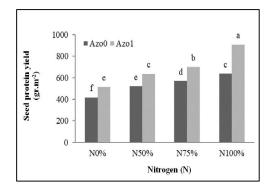


Fig. 11. Mean comparison interaction effect of Nitrogen (N), Azospirillum (Azo) and Pseudomonas (Pse) on seed protein yield via Duncan test at 5% probability level.

Pseudomonas and Azospirillum bacteria had a positive and increasing effect on seed yield. The combined application of Azospirillum and 75 kg of nitrogen fertilizer from urea source per hectare increased seed yield by 30.3% and reduced nitrogen fertilizer application by 25%. In fact, the use of biofertilizers while led to the optimal use of biological organic materials and reducing production costs due to the use of chemical fertilizers, also prevents damage to the environment.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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