Study on replacement probability of biofertilizer with chemical fertilizer in bread wheat (Triticum aestivum L)

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Seyed Morteza Azimzadeh and Seyed Javad Azimzadeh: Study on replacement probability of biofertilizer with chemical fertilizer in bread wheat (Triticum aestivum L)

ABSTRACT

In order to study the replacement probability of biofertilizer with chemical fertilizer in bread wheat, Two separated experiments were conducted in Islamic Azad University of Shirvan during 2011-2012 growing season. In experiment 1, 7 levels of nitrogen fertilizer and 4 levels of Nitroxin biofertilizer and in experiment 2, also 7 levels of phosphorus fertilizer with 4 levels of PB-2 biofertilizer, as factorial, based on randomized complete block design conducted in 3 replications. During the growing season and after harvesting, the traits of, biological yield, seed yield, thousand kernel weight, seed per ear, ear/m², ear length, plant height, seed germination and harvest index were recorded. The results showed that Nitroxin biofertilizer increased seed yield at the rate of 5.6% and also increased thousand kernel weight of wheat from 35.8 to 37 gr. Nitrogen fertilizer increased biological and seed yield linearly. Using 300 Kg/h nitrogen fertilizer compared with no fertilizer application, increased biological yield around 41%. Using 250 kg/h nitrogen fertilizer increased seed yield around 30% compared with using no nitrogen fertilizer. Nitrogen fertilizer treatments imposed a significant and linear effect on number of seed/ear in wheat. Effect of nitrogen fertilizer on number of ear/m² was also significant. The highest number of seed/ear/m² observed in treatments of, 150, 200 and 250 Kg/h nitrogen fertilizer. the highest plant height obtained from 300 Kg/h nitrogen fertilizer which increased plant height around 13.5%. PB-2 biofertilizer increased seed yield at the rate of 9%, that is, using 100 gr/h PB-2 increased seed yield from 7259 to 7937 Kg/h. Effect of ammonium phosphate on seed yield was not significant. 100 gr/h PB-2 increased number of seed/ear from 988 to 1129 equal with 14%. 100 gr/h PB-2 biofertilizer comparing with using no biofertilizer increased plant height at the rate of 4% from 85 to 88 cm. Overall using 200 kg/h nitrogen fertilizer with 2 L/h Nitroxin and 250 kg/h ammonium phosphate with 100gr/h PB-2 biofertilizer are the best combination of chemical and biofertilizer in these experiments.

Key words: Biofertilizer, Nitrogen, Phosphorous, yield, wheat

Introduction

Chemical fertilizers usage are very important in agricultural production. In spite of considerable increase in farmers income, chemical fertilizers created a lot of environmental and ecological problems in last decades [6]. In Iran as other countries, lots of entities consumption of chemical fertilizers in order to gain high yield and compensate deficient of food materials in soil they have destroyed water and soil resources. Besides, leaching of chemical fertilizers which are dissolved in water, specially nitrogen fertilizers have caused contamination in drinking water and soil oritification of lakes and ponds water in some regions [2]. So, scientists have done a lot of study in previous years to evaluate the probability of biofertilizer replacement with chemical fertilizer [9, 19, 20]. The aim of this experiment was also study on the effect of Nitroxin and Phosphate varar-2 biofertilizer on yield and yield components of wheat and the possibility of biofertilizer replacement with chemical fertilizer.

Most of bacteria which are used as biological fertilizers included genera of Azotobacter, Azosporilium, Pseudomonas and Bacillus [24]. Their useful impacts are Phytohormone, Cydrophore, Antibiotic production and also biological nitrogen fixation [3]. Azotobacter, Pseudomonas and Bacillus bacteria can produce some production as Cydrophore. Cydrophores are Chelates or organic components with low molecular weight and can create a complex with some cations like iron. Plants can use cydrophores to provide iron [1].

Nezarat and Gholami [11], evaluated the effect of Azosporilium and Pseudomonas on corn and reported that the absorption ratio of nitrogen, phosphorus, potassium, Iron, Zink and copper elements increased significantly with application of...
bacteria. They explained, that simultaneous application of biofertilizer was more effective in comparison with their individual use.

Biological fertilizers usage specially in intensive farming and poor soils is an unavoidable necessity for retaining the qualitative value of the soil [17, 19]. Bashan et al. [3] showed that biofertilizer application, specially Azospirillum can improve plant dry matter, food material absorption, plants height, leaf size and cereal root length. Rezvan Beidokhti [17], evaluated the effects of Pseudomonas bacterium on wheat and reported that spike number, seed number, thousand kernel weight, seed yield and dry matter were significantly affected by different levels of bacterium, phosphorus fertilizer, and their interaction. Shahsavari et al. [23] reported thousand kernel weigh increment of wheat by Pseudomonas bacterium application. Bacterium application also increased the seed yield of wheat about 9/18 % in comparison with no bacterium application. They reported no significant effect of bacterium on ear length. Yazdani et al. [25], evaluated the effect of seeds from different levels of nitrogen fertilizer on Sayons wheat and reported that seeds from treated plants with 240 kg/h urea fertilizer showed better germination properties rather than other seeds which treated with urea fertilizer, so that these seeds in comparison with non-treated seeds with urea fertilizer had 33% more germination and 36% more germination index. Seeds treated with 360 kg/h nitrogen fertilizer had the lowest percentage of germination speed and germination index.

They also reported that the interaction effect of drought levels and kind of biological fertilizer on wheat germination percentage was significant, while treated seeds with Nitragin, Biophosphorus and no biofertilizer, didn’t have significant difference in non stress condition. With increasing of drought stress, positive effects of bacteria inoculation were observable. Plants under the treatments with biological fertilizer of Nitragin in drought tension levels of -4 and -8 bar had more germination percentage rather than other inoculated seeds. Other scientists have reported that biological fertilizers in the tension conditions such as drought or salt stress can increase the resistance of plants against the environmental stress [2, 17, 22]. Roshdi et al. [18], in a research on Sunflower reported that the biological fertilizers of Biosolphure, Azotobacter and Nitroxin can not afford completely the food elements needed for this plant. If they are used with chemical fertilizers based on the results of soil test, can be effective in increasing seed yield and yield components. Ojaghlou et al. [12], reported that the biological fertilizers of PB-2 and Azotobacter can be useful in providing the food elements like, phosphorus and nitrogen for plants. They also explained that these biofertilizers can’t afford completely the food elements for Safflower but if they are used with chemical fertilizer can increase the yield to 24%. Saleh –Rastin [19], reported the acceleration in corn seed germination under the effect of Azotobacter usage. In another experiment, the simultaniuos effect of Azotobacter and Asosprilum and different levels of nitrogen fertilizer on growth and yield of wheat was evaluated and reported that the seed yield of wheat in check treatment, inoculation of seed with azosprilum, Azotobacter and simultaniuos inoculation of Azotobacter and Asoserilum was 25.7, 25.8, 17.7, 18.8 gr per each vase respectively [15]. Mirshekari and Baser [10], evaluated the effect of Sesame and Rape seed inoculation with Nitragin and reported that when the seed of Rape seed planted after adding 2% of Nitragin, radical and seedling length increased 110 and 60% respectively in comparison with check. Radicle length of Sesame, increased with inoculation of the seed with 4% Nitragin about 48%. It has been reported that Nitroxin has the most effect on thousand kernel weight, plant dry weight, and shoot weight of Chickpea [21]. Biofertilizer consumption, increases the resistance of plant against environmental stress and also compensate the missed microorganism [5]. Piraste- Anosheh [14], evaluated the effect of biofertilizer on Sunflower and reported that the most seed yield gained from biofertilizer and the most plant height gained from chemical fertilizer. According to Green Bioteck Company explanation [26], average of seed yield of wheat was 4216 kg/h with usage of phosphorus chemical fertilizr, while it has increased to 4604 kg/h with the usage of PB-2 biofertilizer. Generally, average of seed yield increment with PB-2, was 420 kg/h and equal with 11.3%. The most effect of biofertilizer is reported in Azarbayjan (21.8%), in Khorasan (18.1%), and in Golestan (13.9%). A lot of study has been conducted on the effect of nitrogen fertilizer on crop yield. El-Din [5], reported that application of nitrogen fertilizer increased seed yield of Triticale. Lack et al. [8], reported that increasing the nitrogen application, increased seed yield linearly. They explained that increasing in number of seed per ear are effective in this case. In another experiment nitrogen fertilizer, increased number of ear/m² [13]. Borjian and Emam [4], reported that spraying urea fertilizer, increased wheat seed yield significantly through increasing number of seed per ear. Khoorgami and Bour [7], also reported that effect of nitrogen fertilizer on plant height, TKW, seed yield and biological yield was significant.

Materials And Methods

In order to study the replacement probability of biofertilizers with chemical fertilizers in bread wheat (Triticum aestivum L), Two separated experiments were conducted in Islamic Azad University of Shirvan during 2011-2012 growing season. In experiment 1, 7 levels of nitrogen fertilizer and 4 levels of Nitroxin biofertilizer as factorial based on
randomized complete block design conducted in 3 replications. The levels of nitrogen fertilizer included, 0, 50, 100, 150, 200, 250 and 300 kg/h, from source of urea. Nitroxin biofertilizer levels included, 0, 2, 4 and 6 L/h. Usage of Nitroxin was seed inoculation.

In experiment 2, also 7 levels of phosphorus fertilizer and 4 levels of PB-2 biofertilizer as factorial based on randomized complete block design conducted in 3 replications. The levels of phosphorus fertilizer included, 0, 50, 100, 150, 200, 250 and 300 kg/h, from source of ammonium phosphate. The levels of PB-2 biofertilizer included, 0, 100 gr/h (at the time of sowing), 100+ 100, (100 gr/h at the time of sowing and 100 gr/h in spring) and 200 gr/h, (at the time of sowing). This experiment also conducted as factorial based on complete block design with 3 replications. In both experiment length and width of plot was 3 and 1.5m respectively. Distance between replications was 2m and distance between plots were 0.5m. Land preparation include, moldboard plow, disk, levelling and making the plots. The using variety was Sayons and the seed rate was based on 300 kg/h. Planting method was by hand and after broadcasting the seed on the plots, they covered by almost 5 cm of soil. To avoid probability effect of fungicide on biofertilizer, seed did not inoculated with fungicide. In experiment 1, simultaneously with planting, 200 kg/h ammonium phosphate broadcasted on plot with hand and mixed with soil. In experiment 2, 250 kg/h nitrogen fertilizer from source of urea, applied in two stages. 50% of urea at planting and 50% at beginning of plant jointing. Both experiment irrigated immediately after planting and second irrigation was done 10 days after first irrigation. From April 3, every 10 day one irrigation was done. Method of irrigation was by polyethylene pipe under the plant canopy on soil surface. Weeding was done by hand in beginning of jointing stage. During the growing season and after harvesting, the traits of, emergence, plant height, ear length, number of seed per ear, thousand kernel weight, biological yield and seed yield was recorded. The collected data analyzed with software of, Excel and Mstatc.

Effective material of Nitroxin are a group of nitrogen stabilizer bacteria from Azospirillum and Azotobacter. Number of spors per gram are 10^8 of each bacterium per milliliter of Nitroxin. PB-2 biofertilizer contains two kinds of phosphate – solubilizing bacterium of Bacillus lentus and Pseudomonas poetida which make the unabsorbable phosphorous compound absorbable by two methods of organic acid and phosphates acid exudation. These bacteria are able to tolerate a wide range of PH between 5-11, high temperature to 42˚C and salt to 3.5% very well. These features have caused the widespread application of this biofertilizer in different soil and crops.

### Results and Discussion

#### Experiment 1:

Effect of nitrogen fertilizer on biological and seed yield was significant (p≤1%) (Table 2). Nitroxin biofertilizer did not show significant effect on biological yield but it's effect on seed yield was effective (p≤0.7%). As shown in fig 1 nitrogen fertilizer increased biological yield linearly. The lowest biological yield observed in treatment with no nitrogen fertilizer, and the highest biological yield produced in treatment with 300 Kg/h nitrogen fertilizer. In spite of no significant difference between 150, 200, 250 and 300 Kg/h nitrogen fertilizer, Using 300 Kg/h nitrogen fertilizer compared with no fertilizer application, increased biological yield around 41%. Khoorgami and Bour [7], also reported significant effect of nitrogen fertilizer on biological yield.

Effect of nitrogen fertilizer on seed yield was also significant (p≤1%). Nitrogen fertilizer as in biological yield increased significant and linearly the seed yield. The treatments of 200, 250 and 300 Kg/h nitrogen fertilizer produced more seed yield than other treatments. With no nitrogen fertilizer application, seed yield was 6198 Kg/h, but application of 300 Kg/h nitrogen fertilizer increased seed yield to 8347 Kg/h, equal with 35% (Fig 2). In spite of no significant difference, seed yield in 200 Kg/h nitrogen fertilizer in comparison with 150 Kg/h nitrogen fertilizer showed 14.5% yield increment and using 300 kg/h nitrogen fertilizer compared with 200 kg/h showed only 3.3% yield increment. So, for seed production, using 200 Kg/h nitrogen fertilizer is advisable because of 30%, 22.9%, 18.7% and 14.5% yield increment compared with treatments of, 0, 50, 100 and 150 Kg/h nitrogen fertilizer. The same results reported by other scientists [5,8,9].

<p>| Table 1: Results of soil analysis in experiment location |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>B</th>
<th>Mn</th>
<th>Zn</th>
<th>Fe</th>
<th>K</th>
<th>P</th>
<th>N</th>
<th>O.C</th>
<th>EC</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppm</td>
<td>Ppm</td>
<td>Ppm</td>
<td>Ppm</td>
<td>Ppm</td>
<td>Ppm</td>
<td>Ppm</td>
<td>Ppm</td>
<td>%</td>
<td>D.s/m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.52</td>
<td>0.58</td>
<td>2.74</td>
<td>0.36</td>
<td>1.58</td>
<td>305</td>
<td>5.58</td>
<td>0.036</td>
<td>0.69</td>
<td>1.314</td>
<td>7.76</td>
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</tr>
</tbody>
</table>

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Fig. 1: Effect of nitrogen fertilizer on biological yield

Table 2: Analysis of variance of recorded traits in wheat under the effect of Nitrogen and Nitroxin in experiment 1

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>Df</th>
<th>Biological yield. Kg/h</th>
<th>Seed yield. Kg/h</th>
<th>T.K.W</th>
<th>Seed/ear</th>
<th>Ear/m²</th>
<th>Ear length-cm</th>
<th>Plant height-cm</th>
<th>Seed germination</th>
<th>HI-%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroxin</td>
<td>3</td>
<td>22720</td>
<td>2684569.0</td>
<td>6.165*</td>
<td>23.541*</td>
<td>10840.8</td>
<td>0.463</td>
<td>2.114</td>
<td>22.76</td>
<td>26.990</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>6</td>
<td>78832956.7</td>
<td>9205482.0</td>
<td>1.446</td>
<td>47.115*</td>
<td>26935.5</td>
<td>0.317</td>
<td>217.201*</td>
<td>371.6</td>
<td>42.952</td>
</tr>
<tr>
<td>Nitroxin*</td>
<td>18</td>
<td>3307097</td>
<td>1086963.8</td>
<td>0.463</td>
<td>5.140</td>
<td>5967.8</td>
<td>0.197</td>
<td>16.873</td>
<td>456</td>
<td>54.07</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>54</td>
<td>4126015</td>
<td>1093185.0</td>
<td>5.976</td>
<td>10479</td>
<td>0.341</td>
<td>11.935</td>
<td>775.5</td>
<td>32.918</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>61</td>
<td>19.8</td>
<td>3.98</td>
<td>9.55</td>
<td>3.03</td>
<td>14.28</td>
<td>9.88</td>
<td>19.8</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

*, **, *** In order significant at the 5% and 1% level of probability

Table 3: Analysis of variance of recorded traits in wheat under the effect of phosphorous and PB-2 in experiment 2

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>Df</th>
<th>Biological yield. Kg/h</th>
<th>Seed yield. Kg/h</th>
<th>T.K.W</th>
<th>Seed/ear</th>
<th>Ear/m²</th>
<th>Ear length-cm</th>
<th>Plant height-cm</th>
<th>Seed germination%</th>
<th>HI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB-2</td>
<td>3</td>
<td>5934044.4</td>
<td>2635650.0</td>
<td>0.513</td>
<td>6.017</td>
<td>1708241.0</td>
<td>0.151</td>
<td>47.46**</td>
<td>2194.2*</td>
<td>3.6</td>
</tr>
<tr>
<td>Ammonium phosphate</td>
<td>6</td>
<td>4139085.7</td>
<td>1202722.0</td>
<td>0.931</td>
<td>2.282</td>
<td>1012548.0</td>
<td>1.534*</td>
<td>20.381</td>
<td>231.3</td>
<td>7.2</td>
</tr>
<tr>
<td>PB-2*</td>
<td>18</td>
<td>4642696.2</td>
<td>1252106.3</td>
<td>0.966</td>
<td>7.553</td>
<td>928425.4</td>
<td>0.489</td>
<td>8.73</td>
<td>367.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Ammonium phosphate</td>
<td>54</td>
<td>3068229.9</td>
<td>1028225.0</td>
<td>0.960</td>
<td>7.006</td>
<td>960737.0</td>
<td>0.381</td>
<td>11.96</td>
<td>640.55</td>
<td>5.1</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>8.9</td>
<td>13.6</td>
<td>10.2</td>
<td>12.3</td>
<td>10.6</td>
<td>4</td>
<td>19.8</td>
<td>6</td>
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</tr>
</tbody>
</table>

*, **, *** In order significant at the 5% and 1% level of probability

Fig. 2: Effect of nitrogen fertilizer on seed yield
Effect of Nitroxin biofertilizer shown in fig 3. As presented Nitroxin biofertilizer at the rate of 2 L/h, in comparison with using no biofertilizer increased seed yield up to 5.6%. Using 4, an 6 L/h, Nitroxin biofertilizer decreased seed yield. Rai and Gaur [15], also reported seed yield increment when sown seed, inoculated with Azotobacter and Azospirillum.

Effect of Nitroxin on thousand kernel weight was also significant (p≤1%) (Table 2). Using 2 L/h, Nitroxin biofertilizer compared with using no biofertilizer increased thousand kernel weight of wheat from 35.8 to 37 gr. Using 4 and 6 L/h, Nitroxin showed no significant effect on wheat thousand kernel weight (fig 4). Thousand kernel weight increment by Nitroxin biofertilizer reported by Shah-Hosaini et al [21], as well. Effect of nitrogen fertilizer on thousand kernel weight was not significant.

![Fig. 3: Effect of Nitroxin biofertilizer on seed yield](image)

![Fig. 4: Effect of Nitroxin biofertilizer on thousand kernel weight](image)

Nitroxin biofertilizer significantly (p≤5%) reduced number of seed per ear in wheat (Table 2). Application of 2L/h, Nitroxin in comparison with using no biofertilizer, decreased number of seed/ear from 27.5 to 25.5. Using 4 and 6 L/h biofertilizer was not different with using no biofertilizer (fig 5). According to thousand kernel weight increment with using 2 L/h Nitroxin biofertilizer, reduction of seed number per ear can be resulted from compensatory effect of yield components of wheat [16].

![Fig. 5: Effect of Nitroxin biofertilizer on number of seed/ear](image)
Effect of nitrogen fertilizer on seed number per ear was significant (p≤1%) (Table 2). As presented in fig 6 nitrogen fertilizer treatments imposed a significant and linear effect on number of seed/ear in wheat. The highest seed in ear observed in 300 Kg/h nitrogen fertilizer treatment (30 seed in ear). Seed increment in ear under the effect of nitrogen fertilizer reported by Lack et al [8] as well. They believed that seed yield increment in wheat under the effect of nitrogen fertilizer is based on increasing of seed number in ear.

Fig. 6: Effect of nitrogen fertilizer on seed/ear

Effect of nitrogen fertilizer on number of ear/m² was significant (p≤5%) (Table 2). The highest number of ear/m² observed in treatments of, 150, 200 and 250 Kg/h nitrogen fertilizer that in order were, 1027, 1012 and 1039 kg/h. Nitrogen fertilizer application more than 250 Kg/h decreased ear/m² to 953 (fig 7). Using 150 Kg/h nitrogen fertilizer compared with using no fertilizer, using 50 Kg/h and using 100 Kg/h, increased number of ear/m² at the rate of 5, 13 and 8% respectively. Increasing number of ear/m² under the effect of nitrogen fertilizer reported by Papastylianou [13].

Fig. 7: Effect of nitrogen fertilizer on number of ear/m²

Nitrogen fertilizer was significantly (p≤1%) effective on plant height (Table 2). Using 100 Kg/h nitrogen fertilizer in comparison with using no fertilizer, increased plant height around 9% from 80 to 87.5 cm. There were no significant different between treatments of 100, 150, 200, 250 and 300 Kg/h nitrogen fertilizer but the most plant height obtained from 300 Kg/h nitrogen fertilizer which increased plant height around 13.5% (fig 8). Khourgami and Bour [7], also reported significant effect of nitrogen fertilizer on plant height.

Fig. 8: Effect of nitrogen fertilizer on plant height
Effect of Nitroxin biofertilizer on number of germinated seed after 20 days was significant (p≤5%) (Table 2). According to fig 9, Nitroxin biofertilizer application unexpectedly decreased number of germinated seed in square meter. When using no Nitroxin biofertilizer, number of germinated seed in square meter was 154 that with using 2 L/h nitroxin showed 11% reduction in seed germination and reached to 137 seedling/m². More reduction in germinated seed observed in treatment of 6 L/h Nitroxin. This result is apposite with Saleh-Rastin [20]. He reported higher seed germination under the effect of Nitroxin biofertilizer application in Corn.

![Fig. 9: Effect of Nitroxin biofertilizer on plant germination](image)

**Experiment 2:**

Effect of PB-2, biofertilizer and ammonium phosphate on biological yield was not significant but effect of PB-2 biofertilizer was effective on seed yield (p≤6%) (Table 3). PB-2 biofertilizer increased seed yield at the rate of 9%, that is, using 100 gr/h PB-2 increased seed yield from 7259 Kg/h to 7937 Kg/h. Other PB-2 treatments (100 + 100 and 200 gr/h) did not show significant effect on seed yield (fig 10). Higher wheat seed yield under the effect of PB-2 biofertilizer reported by Shahsavari et al [23]. They reported an increasing of 9.18% in seed yield of wheat because of seed inoculation by Pseudomonas.

![Fig. 10: Effect of PB-2 biofertilizer on seed yield](image)

Comparing mean seed yield by Duncan’s multiple range test under the effect of ammonium phosphate is shown in fig 11. As shown the highest seed yield obtained by application of 250 Kg/h ammonium phosphate, an increasing at the rate of 6.7% in comparison with no ammonium phosphate fertilizer. Although the interaction effect of PB-2 biofertilizer and ammonium phosphate on seed yield was not significant but the highest seed yield gained when using 250 kg/h ammonium phosphate with 100 g/h PB-2 biofertilizer (8586 kg/h) that in comparison with using 250 kg/h ammonium phosphate with no
PB-2 biofertilizer showed 16% increment in seed yield and in comparison with using 100gr/h PB-2 biofertilizer with no ammonium phosphate showed only 3.5% seed yield increment. This result indicated that PB-2 biofertilizer individually can not provide plant nutrition and it is better to apply with phosphate chemical fertilizer. This result explained by other Scientists. Roshdi et al. [18], in experiment on Sunflower reported that bio fertilizer solely can not compensate plant nutrition. Ojagho et al. [12], also reported the same result in Safflower and emphasized that biofertilizer had better to use accompanied with chemical fertilizer.

**Fig. 11:** Effect of ammonium phosphate on seed yield

Effect of PB-2 on number of ear/m² was significant (p≤1%) (Table 3). As shown in fig 12 100 gr/h, PB-2 increased number of seed/ear from 988 to 1129 equal with 14%. Using 200gr/h PB-2, and using PB-2 in two stages (100+100), did not show significant effect on the number of ear/m². Rezvan Beidokhti [17], also reported increasing of ear/m² by using of biofertilizer.

**Fig. 12:** Effect of PB-2 biofertilizer on number of ear/m²

Plant height affected significantly (p≤5%) from PB-2 biofertilizer (Table 3). As shown in fig 13, 100 gr/h PB-2 biofertilizer comparing with using no biofertilizer increased plant height at the rate of 4% from 85 to 88 cm. other PB-2 biofertilizer treatments did not show significant effect on plant height. Plant height increment with PB-2 biofertilizer reported in another experiment [17]. Effect of ammonium phosphate on plant height was not significant.
Fig. 13: Effect of PB-2 biofertilizer on plant height

PB-2 biofertilizer effect on seed germination was significant (p≤5%) (Table 3). Using 100 gr/h PB-2 and using 200 gr/h in two stages (100+100) compared with using 200 gr/h increased seed germination 3.6 and 8.9% respectively. Application of 200 gr/h biofertilizer decreased number of germinated seed.

Overall as a general conclusion as explained, effect of nitrogen fertilizer on seed yield was significant. Seed yield difference between treatments of 200, 250 and 300 kg/h nitrogen fertilizer was not significant but seed yield in 200 kg/h nitrogen fertilizer compared with 150, 100, 50 and 0 kg/h showed an increment of 14.5%, 18.7%, 22.9% and 30% respectively.

Using 2 L/h Nitroxin biofertilizer increased seed yield at the rate of 5.6% compared with using no biofertilizer.

Interaction effect of Nitroxin and nitrogen fertilizer on seed yield was not significant but according to no significant difference between 200, 250 and 300 kg/h nitrogen fertilizer, it seems that using 200 kg/h nitrogen fertilizer with 2 L/h Nitroxin biofertilizer is a suitable combination of chemical and biofertilizer. Seed yield was 8433 kg/h with using 200 kg/h nitrogen fertilizer and 2 L/h Nitroxin biofertilizer. This combination produced 4.5% seed yield higher than the time that applied 200 kg/h nitrogen fertilizer and using no biofertilizer and 25.8% higher than the time that applied 2 L/h Nitroxin biofertilizer with no nitrogen biofertilizer.

In case of ammonium phosphate and PB-2, the highest seed yield gained when using 250 kg/h ammonium phosphate with 100 g/h PB-2 biofertilizer (8586 kg/h) that in comparison with using 250 kg/h ammonium phosphate with no PB-2 biofertilizer showed 16% increment in seed yield and in comparison with using 100gr/h PB-2 biofertilizer with no ammonium phosphate showed only 3.5% seed yield increment.

These results indicated that Nitroxin and PB-2 biofertilizer individually can not provide plant nutrition and it is better to apply with nitrogen and phosphate chemical fertilizer. This result explained by other Scientists. Roshdi et al [18] in experiment on Sunflower reported that biofertilizer solely can not compensate plant nutrition. Ojaghloo et al [12], also reported the same result in Safflower and emphasized that biofertilizer had better to use accompanied with chemical fertilizer. According to results of theses experiments, using 200 kg/h nitrogen fertilizer with 2 L/h Nitroxin biofertilizer and using 250 kg/h ammonium phosphate with 100 gr/h PB-2 are more suitable than other combination and biofertilizer individualy can not replaced with chemical fertilizer.

References