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Assessment Effect of Nitroxin and Phosphorus Biofertilizer on Faba Bean (*Vicia faba* L.) Crop Production, Seed Protein Content and Correlation between Traits

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## ABSTRACT

**BACKGROUND:** Nutrient management plays an great role for obtaining satisfactory yields and to increase crop productivity. It may be achieved by the involvement of organic sources, biologic fertilizers, and micro-nutrients.

**OBJECTIVES:** The current study was done to investigate the effect of application of biofertilizers containing nitrogen and phosphorus biologic fertilizer on some quantitative and qualitative parameters of Faba bean and relation between measured traits.

**METHODS:** This research was carried out via factorial experiment based on randomized complete blocks design with four replications along 2013 year. The treatments included Nitroxin biologic fertilizer (N<sub>1</sub>: nonuse of Nitroxin or control, N<sub>2</sub>: 250 cc.1000m<sup>2</sup>, N<sub>3</sub>: 500 cc.1000m<sup>2</sup>) and phosphorus biofertilizer (P<sub>1</sub>: nonuse of Phosphorus biofertilizer or control, P<sub>2</sub>: 100 gr.ha<sup>-1</sup>, P<sub>3</sub>: 150 gr.ha<sup>-1</sup>).

**RESULT:** According result of analysis of variance effect of different level of Nitroxin and phosphorus biofertilizer on all studied traits was significant. Also interaction effect of treatments on number of seed per pod, number of pod per plant and plant height was not significant. Evaluation mean comparison result of interaction effect of treatments on all measured traits revealed the highest amount of seed yield (5096 kg.ha<sup>-1</sup>), biologic yield (9230 kg.ha<sup>-1</sup>), 1000-seed weight (1450 gr.ha<sup>-1</sup>) Seed protein percentage (24%), leaf area index (4.5) and harvest index (53.2%) were noted for N<sub>3</sub>P<sub>3</sub> treatment and lowest amount of mentioned traits belonged to control. The most significant correlation between seed yield and Biologic yield (r=0.95<sup>\*\*</sup>), number of pod per plant (r=0.81<sup>\*\*</sup>), 1000-seed weight (r=-0.73<sup>\*\*</sup>), number of seed per pod (r=0.68<sup>\*\*</sup>) and Harvest index (r=0.67<sup>\*\*</sup>), respectively was observed.

**CONCLUSION:** Finally according result of current research application 500 cc.1000m<sup>2</sup> Nitroxin and 150 gr.ha<sup>-1</sup> Phosphorus biofertilizer had the highest amount of studied traits and it can be advice to producers in studied region.

**KEYWORDS:** Leaf area index, Nitrogen, Nutrition, Pulse, Yield.

## 1. BACKGROUND

Faba bean is one of the oldest crops grown by human that is used as a source of protein in diets, as fodder and a forage crop for animals, and for available nitrogen in the biosphere (Kopke and Nemecek, 2010). The Faba bean contributes to the sustainability of cropping systems via: (1) its ability to contribute nitrogen (N) to the system via biological N<sub>2</sub> fixation, (2) diversification of systems leading to decreased disease, pest and weed build-up and potentially increased biodiversity, (3) reducing the consumption of fossil energy in plant production, and (4) providing food and feed rich in protein (Jensen et al., 2010). Faba bean is distributed in more than 55 countries with harvesting area of 2.56 million ha and totally yield of 4.56 million tons of dry grain. Asia and Africa accounted for 72% of the area and 80% of the production of dry Faba bean grains (FAOSTAT, 2012). Faba bean is one of the main crops grown in Golestan province (northeast of Iran), with harvesting area of 1838 ha and yield of approximately 7056 tons dry grain in 2010 (Agriculture Organization of Golestan Province, 2011). After the industrial revolution widespread introduction of inorganic fertilizers led to a decline in the use of organic material in the cropping systems (Hasanozaman et al., 2010). Nutrient management plays an important role for obtaining satisfactory yields and to increase crop productivity. It may be achieved by the involvement of organic sources, bio fertilizers, and micro-nutrients (Singh et al., 2002). The amount of growth and photosynthetic translocation is related to

nutrients availability (Munir et al., 2012). Studies have shown that longterm use of fertilizers reduces crop yields. This decrease is due to the acidification of the soil texture, the reduction of biological activity of the soil and the inappropriate physical properties of the soil (Alexandratos, 2003). Beyranvand et al. (2013) suggested that effect of nitrogen and phosphate biological fertilizers were evaluated the positively, there were an increase in plant height, ear weight, and number of grain per cob, grain yield and biomass yield. Increasing yield was attributed to the plant growth promoting substances by root colonizing bacteria more than the biological nitrogen fixation, stated that yield increased due to promoting root growth which in turn enhancing nutrients and water uptake from the soil (Lin et al., 1983). The response of cereals to inoculations with the Nitrokara is different in terms of bacterial strains and soil and weather conditions in the region and in positive response cases, the increase in the product is reported to be about 7 to 12% and up to a maximum of 39% (Khavari, 2010). Nitroxin contains nitrogen fixation bacteria (Azotobacter) not only fixes the air nitrogen and balance the uptake of macronutrients and micronutrients but also enhances plant growth and increase the quality and quantity of products through the synthesis and secretion of growth promoting substances (Ansari and Rousta, 2008). Nitroxin biological fertilizers include a series of nitrogen-fixing bacteria of the genus Azotobacter, and Azospirillum that causes the growth of roots and aeri-

al parts of the plant (Gilik et al., 2001). Nouraki et al. (2016) reported the bacteria have positive role in the production of biological fertilizers and hormones which play a significant and great role in regulating process of the plant growth while mixing them with the chemical fertilizers as a supplement the level and depth of the roots. This combination also increases the rate of water and nutrient absorbance which raise the rate of growth and photosynthesis. These combination also increase the seed yield, its components, and biological function, it has been found that biological fertilizers can be combined with the chemical fertilizers in a complementary way to reduce the excessive amount of chemical fertilizers used to grow corn. So the mixing of bio fertilizer with chemical fertilizers could reduce the needs of chemical fertilizers up to 25%. The bacteria in the Nitroxin biological fertilizer, in addition to stabilizing nitrogen elements of the air and balancing the absorption of macronutrient and the micronutrient elements, stimulate growth of the hormones by synthesizing and securing growth promoters such as hormones, although the Nitroxin component produced and supplied with the approval of the country's research institutes (Fulchirri and Frioni, 1994; Asadi Kupaland and Zadeh Laserjan, 2009).

## 2. OBJECTIVES

The current study was done to investigate effect of use of bio-fertilizers containing nitrogen and phosphorus biologic fertilizer on some quantitative and qualitative parameters of Faba bean and relation between measured traits.

## **3. MATERIALS AND METHODS**

## 3.1. Field and Treatments Information

This research was carried out via factorial experiment based on randomized complete blocks design with four replications along 2013 year. Place of research was located in Behbahan city at longitude 50°14'E and latitude 30°36'N in Khuzestan province (Southwest of Iran). The treatments included Nitroxin biologic fertilizer (N<sub>1</sub>: nonuse of Nitroxin or control, N<sub>2</sub>: 250 cc.1000m<sup>2</sup>, N<sub>3</sub>: 500 cc.1000m<sup>2</sup>) and phosphorus bio-fertilizer (P<sub>1</sub>: nonuse of Phosphorus biofertilizer or control, P<sub>2</sub>: 100 gr.ha<sup>-1</sup>, P<sub>3</sub>: 150 gr.ha<sup>-1</sup>).

## 3.2. Farm Management

This experiment had 36 plots. Each plot consisted of 6 lines with a distance of 50 cm and 5 meters length. The distance between the shrubs on every row was 10 cm. To determine the soil properties and the physical and chemical characteristics was mentioned in table 1.

Table 1. Physical and chemical properties of the field soil (depth: 0-30 cm)

Tissue	Clay (%)	Silt (%)	Sand (%)	Potassium (ppm)	Phosphorus (ppm)	Total Nitrogen (%)	O.C (%)	E.C (ds.m <sup>-1</sup> )	рН
Loam	16	34	50	240	12	0.201	2.3	1.25	7.9

For mix seeds with bio-fertilizer of Nitroxin (Prepared by Asian love Biotechnology Company), seed was first spread on a clean plastic. Then, the appropriate amount of inoculum (1 liter per 60 kg of seed) was gradually sprayed on the seeds and by seeding the seeds inoculum was done. Then the inoculated seeds were shaded and immediately after drying, seeds planted (Akabarynia, 2004). To apply phosphorus biofertilizer, the biofertilizer (100 g.ha<sup>-1</sup>) was dissolved in a 10 liter container filled with water. Then the seeds were placed in these containers for 10 minutes before planting and impregnated with fertilizer solution and then they were planted. Phosphorus biofertilizer (brand Barvar 2) contains  $10^7$  to  $10^8$  phosphatesolubilizing bacteria (Pantoea agglomerans strain P5 and Pseudomonas strain P13) per gram of product, which are released phosphates ions by producing organic acids and phosphatase enzymes around the roots. During the planting, separate disposable gloves for each treatment were used to prevent mixing of the effects of treatments. Other fertilizers were used as the base. Seeds were planted manually with the distance of 10 cm and at the depth of 5 cm in November 5, 2013. The first irrigation was done in November 7, 2013. The next irrigations were done based on plant need, temperature, and atmospheric conditions. To combat the weeds during the growth, weeding and thinning was done manually in December 19, 2013. After the leaves got yellow and the pods changed from green to brown color, the harvest operation was carried out.

## 3.3. Measured Traits

After physiological ripening seed yield, biologic yield, number of pods per plant, number of seed per pod, 1000-seed weight and plant height was determined. To determine the percentage of seed protein, the percentage of grain nitrogen was first measured by Kjeldahl method, which includes digestion, distillation and titration. To measure the amount of seed protein by multiplying the percentage of seed nitrogen by a factor of 6.25, the amount of protein in the seed was obtained. Then, by multiplying the percentage of protein in each treatment by its seed yield, the protein yield for each treatment was calculated (Keeney and Nelson, 1982). Leaf area index was measured by leaf area meter AccuPAR (Model L.P80). Harvest index (HI) was calculated according to formula of Gardener et al. (1985) follows: Equ.1. HI= (Seed as yield/Biologic yield) ×100.

## 3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level. Curve was drawn with M.S Office (Excel, Ver. 2010) software.

## 4. RESULT AND DISCUSSION

# 4.1. Number of seed per pod

According result of analysis of variance effect of Nitroxin and Phosphorus biofertilizer on number of seed per pod was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of Nitroxin indicated that maximum number of seed per pod (4.1) was noted for  $N_3$  and minimum of that (3.6) belonged to control treatment (Table 3). As for Duncan classification made with respect to different level of Phosphorus biofertilizer maximum and minimum amount of number of seed per pod belonged to  $P_3$  (3.9) and control (2.7) (Table 4).

S.O.V	df	No. seed per pod	No. pod per plant	1000-seed weight	Seed yield	Biological yield
Replication	3	0.25	0.03	419	3058	1986945
Nitroxin (N)	2	5.1**	53.9**	241008**	4886711**	7034007**
Phosphorus biofertilizer (P)	2	3.7**	35.8**	78508**	3248233**	2367834**
$\mathbf{N} \times \mathbf{P}$	4	0.25 <sup>ns</sup>	1.03 <sup>ns</sup>	9020**	700965**	634235**
Error	24	0.09	0.57	40.5	550	45893
CV (%)	-	8.6	5.4	5.1	6.2	7.7

Table 2. The ANOVA results of Nitroxin and Phosphorus biofertilizer on measured traits

<sup>ns, \* and \*\*</sup>: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.									
<b>S.O.V</b>	df	Harvest index	Leaf area index	Plant height	Seed protein percentage				
Replication	3	2.08	0.001	28.7	0.011				
Nitroxin (N)	2	147**	5.2**	301**	$62^*$				
Phosphorus Biofertilizer (P)	2	152**	1.16**	120**	11.2*				
$\mathbf{N} \times \mathbf{P}$	4	34**	$0.049^{**}$	4.5 <sup>ns</sup>	1.52**				
Error	24	0.59	0.007	1.6	0.27				
CV (%)	-	6.7	5.22	8.71	2.21				

<sup>ns,\* and \*\*</sup>: no significant, significant at 5% and 1% of probability level, respectively.

## 4.2. Number of pod per plant

Result of analysis of variance revealed effect of Nitroxin and Phosphorus biofertilizer on number of pod per plant was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). According result of mean comparison maximum of number of pod per plant (16.5) was obtained for 500 cc.1000m<sup>2</sup> Nitroxin (N<sub>3</sub>) and minimum of that (11.6) was for control treatment (Table 3). Evaluation mean comparison result indicated in different level of Phosphorus biofertilizer the maximum number of pod per plant (15.5) was noted for P<sub>3</sub> and minimum of that (11.7) belonged to control treatment (Table 4).

#### Amini, Assessment Effect of Nitroxin and Phosphorus Biofertilizer...

Treatment No. seed per pod		No. pod per plant	1000-seed weight (gr.ha <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )	Biological yield (kg.ha <sup>-1</sup> )	
N <sub>1</sub>	3.6 <sup>c</sup>	11.6 <sup>c</sup>	1066 <sup>b</sup>	2953°	6859 <sup>b</sup>	
$N_2$	3.7 <sup>b</sup>	13.8 <sup>b</sup>	1326 <sup>a</sup>	3911 <sup>b</sup>	7694 <sup>ab</sup>	
$N_3$	4.1 <sup>a</sup>	16.5 <sup>a</sup>	1368 <sup>a</sup>	4402 <sup>a</sup>	8626 <sup>a</sup>	

Table 3. Mean comparison effect of different level of Nitroxin biofertilizer on measured traits

\*Means with similar letters in each column are not significantly different by Duncan test at 5% probability level.  $N_1$ : nonuse of Nitroxin or control,  $N_2$ : 250 cc.1000m<sup>2</sup>,  $N_3$ : 500 cc.1000m<sup>2</sup>

Continue table 3.									
Treatment	Harvest index (%)	Leaf area index	Plant height (cm)	Seed protein percentage (%)					
$N_1$	43.05 <sup>b</sup>	2.9 <sup>b</sup>	37.5 <sup>b</sup>	18.3 <sup>b</sup>					
$N_2$	50.83 <sup>a</sup>	$4.0^{\mathrm{a}}$	47.1 <sup>a</sup>	22.7 <sup>a</sup>					
$N_3$	51.03 <sup>a</sup>	4.3 <sup>a</sup>	48.0 <sup>a</sup>	23.0 <sup>a</sup>					

\*Means with similar letters in each column are not significantly different by Duncan test at 5% probability level. N<sub>1</sub>: nonuse of Nitroxin or control, N<sub>2</sub>: 250 cc.  $1000m^2$ , N<sub>3</sub>: 500 cc.  $1000m^2$ 

Fallahi *et al.* (2008) founded that Nitroxin bio-fertilizer had significant effects on main yield components, seed yield; essential oil .They concluded that this bio-fertilizer can be considered as a replacement for chemical fertilizers the absorbed nitrogen during this time leads to the increase of the number of spikelet. In different experiments it was observed that the yield and its components increased in the crop inoculated with Azospirillum.

## 4.3. 1000-seed weight

According result of analysis of variance effect of Nitroxin, Phosphorus biofertilizer and interaction effect of treatments on 1000-seed weight was significant at 1% probability level (Table 2). Assessment mean comparison result indicated in different level of Nitroxin the maximum 1000-seed weight (1368 gr.ha<sup>-1</sup>) was noted for N<sub>3</sub> and minimum of that

(1066 gr.ha<sup>-1</sup>) belonged to control treatment (Table 3). Compare different Phosphorus biofertilizer level of showed that the maximum and the minimum amount of 1000-seed weight belonged to P<sub>3</sub> (1320 gr.ha<sup>-1</sup>) and control (1147 gr.ha<sup>-1</sup>) treatments (Table 4). Evaluation mean comparison result of interaction effect of treatments indicated maximum 1000-seed weight (1453 gr.ha<sup>-1</sup>) was noted for  $N_3P_3$  and lowest one (1032 gr.ha<sup>-1</sup>) belonged to control treatment (Table 5). Tarang et al. (2013) reported applications of Nitroxin biofertilizer and chemical fertilizer (400 kg.ha<sup>-1</sup> urea with 300 kg.ha<sup>-1</sup> ammonium phosphate) had a significant effect on traits of root dry weight, number of seed per row (36.5), number of seeds per ear (458.56), 1000-grain weight, seed (13.23 t.ha<sup>-1</sup>), biological yield  $(26.4 \text{ t.ha}^{-1})$  and the harvest index (53.88%).

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Treatment	eatment No. seed per pod		1000-seed weight (gr.ha <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )	Biological yield (kg.ha <sup>-1</sup> )	
<b>P</b> <sub>1</sub>	2.7 <sup>b</sup>	11.7 <sup>c</sup>	1147 <sup>b</sup>	3068 <sup>b</sup>	7136 <sup>b</sup>	
$\mathbf{P}_2$	3.8 <sup>a</sup>	14.7 <sup>b</sup>	1294 <sup>a</sup>	4021 <sup>ab</sup>	7978 <sup>a</sup>	
<b>P</b> <sub>3</sub>	3.9 <sup>a</sup>	15.5 <sup>a</sup>	1320 <sup>a</sup>	4178 <sup>a</sup>	8065 <sup>a</sup>	

Table 4. Mean comparison effect of different level of Phosphorus biofertilizer on studied traits

\*Means with similar letters in each column are not significantly different by Duncan test at 5% probability level. P<sub>1</sub>: nonuse of Phosphorus biofertilizer or control, P<sub>2</sub>: 100 gr.ha<sup>-1</sup>, P<sub>3</sub>: 150 gr.ha<sup>-1</sup>

Continue table 4.								
Treatment	Harvest index (%)	Leaf area index	Plant height (cm)	Seed protein percentage (%)				
<b>P</b> <sub>1</sub>	42.99 <sup>b</sup>	3.3 <sup>b</sup>	40.0 <sup>b</sup>	20.1 <sup>c</sup>				
$\mathbf{P}_2$	50.40 <sup>a</sup>	3.9 <sup>a</sup>	46.5 <sup>a</sup>	21.7 <sup>b</sup>				
<b>P</b> <sub>3</sub>	51.80 <sup>a</sup>	$4.0^{a}$	46.1 <sup>a</sup>	22.2 <sup>a</sup>				

\*Means with similar letters in each column are not significantly different by Duncan test at 5% probability level.  $P_1$ : nonuse of Phosphorus biofertilizer or control,  $P_2$ : 100 gr.ha<sup>-1</sup>,  $P_3$ : 150 gr.ha<sup>-1</sup>

## 4.4. Seed yield

Result of analysis of variance showed effect of Nitroxin, Phosphorus biofertilizer and interaction effect of treatments on seed yield was significant at 1% probability level (Table 2). Evaluation mean comparison result revealed in different level of Nitroxin the maximum seed yield (4402 kg.ha<sup>-1</sup>) was noted for N<sub>3</sub> and minimum of that (2953 kg.ha<sup>-1</sup>) belonged to control treatment (Table 3). Between different levels of Phosphorus biofertilizer the maximum seed yield (4178 kg.ha<sup>-1</sup>) was observed in  $P_3$  and the lowest one (3068 kg.ha<sup>-1</sup>) was found in control treatment (Table 4). Assessment mean comparison result of interaction effect of treatments indicated maximum seed yield (5096 kg.ha <sup>1</sup>) was noted for  $N_3P_3$  and lowest one (2585 kg.ha<sup>-1</sup>) belonged to control treatment (Table 5). In study of Azotobacter and Azospirillum bacteria on corn was found that inoculation with these bacteria increases corn yield (Biari *et al.*, 2011). Application of Nitroxin biological fertilizer in the sesame plant increases number of seeds per capsule, seed weight, biological function, and seed yield (Boraste, 2009).

## 4.5. Biological yield

According result of ANOVA effect of Nitroxin, Phosphorus biofertilizer and interaction effect of treatments on biological yield was significant at 1% probability level (Table 2). Mean comparison of different level of Nitroxin indicated the maximum biological yield (8626 kg.ha<sup>-1</sup>) was obtained for N<sub>3</sub> and minimum of that (6859 kg.ha<sup>-1</sup>) was for control treatment (Table 3). Similar result observed by Tarang et al. (2013), they reported application of 1 L.ha<sup>-1</sup> Nitroxin bio-fertilizer with chemical fertilizer had a strong effect on productivity, serves to reduce environmental pollution and led to achieve maximum amount of biologic yield.

#### Amini, Assessment Effect of Nitroxin and Phosphorus Biofertilizer...

Nitroxin biofertilizer	Phosphorus biofertilizer	No. seed per pod	No. pod per plant	1000-seed weight (gr.ha <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )	Biological yield (kg.ha <sup>-1</sup> )
	<b>P</b> <sub>1</sub>	3.8 <sup>a</sup>	13.8 <sup>a</sup>	1032 <sup>f</sup>	2585 <sup>e</sup>	6720 <sup>d</sup>
$N_1$	$\mathbf{P}_2$	$4.0^{\mathrm{a}}$	13.7 <sup>a</sup>	1071 <sup>e</sup>	3020 <sup>d</sup>	6848 <sup>d</sup>
	<b>P</b> <sub>3</sub>	$4.0^{\mathrm{a}}$	$14.0^{a}$	1095 <sup>e</sup>	3256 <sup>cd</sup>	7010 <sup>cd</sup>
	<b>P</b> <sub>1</sub>	$4.0^{\mathrm{a}}$	13.8 <sup>a</sup>	1188 <sup>d</sup>	3546 <sup>°</sup>	7236 <sup>c</sup>
$N_2$	$\mathbf{P}_2$	$4.0^{\mathrm{a}}$	13.7 <sup>a</sup>	1378 <sup>b</sup>	4006 <sup>b</sup>	7891 <sup>b</sup>
	<b>P</b> <sub>3</sub>	$4.0^{\mathrm{a}}$	$14.0^{a}$	1411 <sup>ab</sup>	4181 <sup>b</sup>	7955 <sup>b</sup>
	<b>P</b> <sub>1</sub>	3.8 <sup>a</sup>	$14.0^{a}$	1220 <sup>c</sup>	3073 <sup>d</sup>	7453 <sup>bc</sup>
$N_3$	$\mathbf{P}_2$	3.8 <sup>a</sup>	$14.0^{a}$	1433 <sup>ab</sup>	5038 <sup>ab</sup>	9196 <sup>ab</sup>
	<b>P</b> <sub>3</sub>	$4.0^{\mathrm{a}}$	13.8 <sup>a</sup>	1453 <sup>a</sup>	5096 <sup>a</sup>	9230 <sup>a</sup>

Table 5. Mean comparison interaction effects of treatment on measured traits

\*Means with similar letters in each column are not significantly different by Duncan test at 5% probability level.

 $N_1$ : nonuse of Nitroxin or control,  $N_2$ : 250 cc. 1000m<sup>2</sup>,  $N_3$ : 500 cc. 1000m<sup>2</sup>

P<sub>1</sub>: nonuse of Phosphorus biofertilizer or control, P<sub>2</sub>: 100 gr.ha<sup>-1</sup>, P<sub>3</sub>: 150 gr.ha<sup>-1</sup>

Continue table 5.								
Nitroxin	Phosphorus	Harvest	Leaf area	Plant height	Seed protein			
biofertilizer	biofertilizer	index (%)	index	(cm)	percentage (%)			
	<b>P</b> <sub>1</sub>	38.3 <sup>d</sup>	2.3 <sup>e</sup>	47 <sup>a</sup>	18.0 <sup>c</sup>			
$N_1$	$\mathbf{P}_2$	42.0 <sup>c</sup>	3.0 <sup>d</sup>	47 <sup>a</sup>	18.2 <sup>c</sup>			
	<b>P</b> <sub>3</sub>	44.2 <sup>bc</sup>	3.3 <sup>d</sup>	$48^{\mathrm{a}}$	18.8 <sup>c</sup>			
	<b>P</b> <sub>1</sub>	46.8 <sup>b</sup>	3.7 <sup>c</sup>	47 <sup>a</sup>	21.0 <sup>b</sup>			
$N_2$	$\mathbf{P}_2$	48.7 <sup>b</sup>	4.1 <sup>ab</sup>	$48^{\mathrm{a}}$	23.3 <sup>ab</sup>			
	<b>P</b> <sub>3</sub>	50.4 <sup>ab</sup>	$4.2^{ab}$	$48^{a}$	24.0 <sup>a</sup>			
	<b>P</b> <sub>1</sub>	39.4 <sup>d</sup>	$4.0^{b}$	47 <sup>a</sup>	21.3 <sup>b</sup>			
$N_3$	$\mathbf{P}_2$	52.8 <sup>ab</sup>	4.3 <sup>ab</sup>	47 <sup>a</sup>	23.6 <sup>ab</sup>			
	<b>P</b> <sub>3</sub>	53.2 <sup>a</sup>	4.5 <sup>a</sup>	47 <sup>a</sup>	$24.0^{a}$			

\*Means with similar letters in each column are not significantly differentt by Duncan test at 5% probability level.

 $N_1$ : nonuse of Nitroxin or control,  $N_2$ : 250 cc. 1000m<sup>2</sup>,  $N_3$ : 500 cc. 1000m<sup>2</sup>

P<sub>1</sub>: nonuse of Phosphorus biofertilizer or control, P<sub>2</sub>: 100 gr.ha<sup>-1</sup>, P<sub>3</sub>: 150 gr.ha<sup>-1</sup>

Vadivel *et al.* (1999) concluded that the application of Nitroxin biological fertilizer had a significant effect on all measured parameters except 1000-grian weight and grain-straw ratio. Grain yield, straw yield, plant height, spike length, number of grains per spike and number of spikes per square meter increased in Nitroxin treatment in comparison to the control treatment. According to (Boraste, 2009) plant height and plant diameter in corn increase much more in the effect of inoculation with *Azotobacter* and *Azospirillum* bacteria than non-inoculated. Besides, inoculation of wheat seeds with bacteria such as *Azotobacter* and *Azospirillum* can lead to stem dry weight, and dry weight of plant (Defreitas, 2000). Compare different level of Phosphorus biofertilizer showed that the maximum and the minimum amount of biological yield belonged to P<sub>3</sub> (8065 kg.ha<sup>-1</sup>) and control (7136 kg.ha<sup>-1</sup>) treatments (Table 4). Evaluation mean comparison result of interaction effect of treatments indicated maximum biological yield (9230 kg.ha<sup>-1</sup>) was noted for N<sub>3</sub>P<sub>3</sub> and lowest one (6720 kg.ha<sup>-1</sup>) belonged to control treatment (Table 5). Azimi *et al.*  (2013a) found that application of super nitroplass bio-fertilizer with Phosphate barvar2 treatment has the highest seed yield (7.6 t.ha<sup>-1</sup>) and non-application of bio-fertilizers treatment has the Pishtaz cultivar has the lowest seed yield (6.3 t.ha<sup>-1</sup>). Azimi *et al.* (2013b) was reported that grain yield and biomass yield increasing with the bio fertilizer application, also which account important benefit, causing decreasing in the inputs of production because of economizing much money to chemical fertilizers and increasing in yield and biological yield.

## 4.6. Harvest index

Result of analysis of variance indicated effect of Nitroxin, Phosphorus biofertilizer and interaction effect of treatments on harvest index was significant at 1% probability level (Table 2). According mean comparison result of different level of Nitroxin the maximum harvest index (51.03%) was observed in  $N_3$  and the lowest one (43.05%) was found in control treatments (Table 3). The variability of the harvest index in the plants depends on the difference in the production of the assimilates during the seed filling and re-transplantation of the assimilates before the pollination of each genotype and the strength of the reservoir (Nour mohammadi et al., 2001). Han and Lee (2006) attributed the increase in corn harvest index in bio-fertilizer treatment to better absorb nutrients. Because the plant with better absorption of nutrients and increasing leaf area index can use better solar radiation and send more photosynthetic materials to seed and thus increase dry matter. Between different levels of

manganese Nano-chelate highest value of harvest index was belonged to the  $P_3$ treatment (51.80%) and the lowest one was found in the control treatment as 42.99% (Table 4). Evaluation mean comparison result of interaction effect of treatments indicated maximum harvest index (53.20%) was noted for  $N_3P_3$ and lowest one (38.30%) belonged to control treatment (Table 5).

## 4.7. *Leaf area index*

According result of analysis of variance effect of Nitroxin, Phosphorus biofertilizer and interaction effect of treatments on leaf area index was significant at 1% probability level (Table 2). Evaluation mean comparison result revealed in different level of Nitroxin the maximum leaf area index (4.3) was noted for  $N_3$  and minimum of that (2.9) belonged to control treatment (Table 3). Rahi (2013) reported that increase in Nitroxin also increased fresh and dry weights of leaf, stem, chlorophylls a, b, total carotenoids, and anthocyanin content of the plants linearly. Garg et al. (2005) reported increasing nitrogen to soil led to increase the plant photosynthetic efficiency and ultimately increased the seed yield and growth rate. On the other hand, since the rate of light absorption by leaves and converting it into photosynthetic materials are the other factors affecting the plant growth and production, the increase of leaf area in the farm leads to the increase of light absorption and ultimately leads to the increase of seed yield. Between different levels of Phosphorus biofertilizer the maximum leaf area index (4.0) was observed in P<sub>3</sub> and the lowest one (3.3) was found in

control treatment (Table 4). Assessment mean comparison result of interaction effect of treatments indicated maximum leaf area index (4.5) was noted for  $N_3P_3$  and lowest one (2.3) belonged to control treatment (Table 5).

## 4.8. Plant height

According result of analysis of variance effect of Nitroxin and Phosphorus biofertilizer on plant height was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of Nitroxin indicated that maximum number of seed per pod (48.0 cm) was noted for N<sub>3</sub> and minimum of that (37.5 cm) belonged to control treatment (Table 3). As for Duncan classification made with respect to different level of Phosphorus biofertilizer maximum and minimum amount of number of seed per pod was for  $P_3$  (46.1) cm) and control (40.0 cm) (Table 4).

## 4.9. Seed protein percentage

According result of analysis of variance effect of the Nitroxin, Phosphorus biofertilizer and interaction effect of treatments on the seed protein percentage trait was significant at 5% and 1% probability level, respectively (Table 2). Also assessment mean comparison result indicated in different level of Nitroxin the maximum seed protein percentage (23.0 %) was noted for N<sub>3</sub> and minimum of that (18.03 %) belonged to control treatment (Table 3). Compare different level of the Phosphorus biofertilizer showed that the maximum and the minimum amount of seed protein percentage belonged to P<sub>3</sub> (22.2%) and control (20.1%) treatments (Table 4). Evaluation mean comparison result of interaction effect of treatments indicated maximum seed protein percentage (24.0%) was noted for  $N_3P_3$  and lowest one (18.0%) belonged to control treatment (Table 5).

## 4.10. Correlation between traits

Simple correlation coefficients between traits these coefficients were estimated according to Pearson coefficient (Table 5). The most significant correlation between seed yield and Biologic yield (r=0.95\*\*), number of pod per plant (r=0.81\*\*), 1000-seed weight (r=-0.73<sup>\*\*</sup>), number of seed per pod  $(r=0.68^{**})$  and Harvest index  $(r=0.67^{**})$ , respectively was observed. Also the traits of leaf area index (r=0.55<sup>\*</sup>), seed protein percentage  $(r=-0.52^*)$  and plant height  $(r=0.51^*)$  had correlation with the seed yield at 5% probability level. Fig. 1. showed a significant relation between seed yield and biologic yield.



Fig. 1. Correlation between seed yield and biologic yield

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Table 6. Correlation between studied traits									
Traits	No. seed per pod	No. pod per plant	1000-seed weight	Seed yield	Biologic yield	Harvest index	Leaf area index	Plant height	Seed protein percentage
No. seed per pod	1								
No. pod per plant	-0.52*	1							
1000-seed weight	-0.55*	$0.68^{**}$	1						
Seed yield	$0.68^{**}$	0.81**	-0.73**	1					
Biological yield	0.79**	$0.82^{**}$	0.75**	0.95**	1				
Harvest index	$0.54^{*}$	0.53*	$0.71^{**}$	0.67**	$0.59^{*}$	1			
Leaf area index	0.39 <sup>ns</sup>	$0.52^{*}$	$0.59^{*}$	$0.55^{*}$	0.81**	0.61*	1		
Plant height	0.41 <sup>ns</sup>	0.35 <sup>ns</sup>	0.33 <sup>ns</sup>	0.51*	0.73**	0.66**	$0.50^{*}$	1	
Seed protein percentage	0.61*	$0.65^*$	-0.76**	-0.52*	$0.57^{*}$	0.55*	0.41 <sup>ns</sup>	0.29 <sup>ns</sup>	1

<sup>ns,\* and \*\*</sup>: no significant, significant at 5% and 1% of probability level, respectively.

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The increase of total dry matter and its direct relation with seed yield show the relations between photosynthesis efficiency of plant and grain yield, therefore varieties which have gained more profit of production factor according to growth conditions and they keep more photosynthesis materials in their sinks, have more efficiency. This status was in conformity with the results of some other researchers (Matlabipor et al., 2000). Correlation coefficient between seed yield and number of pods per plant, is because it is assimilate supplier for the grains, therefore, we can consider the positive and significant correlation of grains per pod, with grain yield, a natural thing. As a result, the more this trait is observed, the bigger sink plant would have for metabolic materials. These results had conformity with other researchers (Jorgeh, 2003). The significant and positive correlation between harvest index and seed yield indicate efficiency and kind of photosynthesis materials distribution in different parts of plant, especially in grain. Results of some other researchers verify the mentioned issues (Rabiee et al., 2004).

## **5. CONCLUSION**

Finally according result of current research application  $500 \text{ cc.} 1000\text{m}^2 \text{ Ni-troxin}$  and  $150 \text{ gr.ha}^{-1}$  Phosphorus biological fertilizer (N<sub>3</sub>P<sub>3</sub>) had the highest amount of studied traits and it can be advice to producers in studied region.

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## FOOTNOTES

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