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## Effect of Bio-Fertilizers Containing Nitrogen and Phosphorus Fixing Bacteria on Yield and Yield Components of Faba Bean

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### ABSTRACT

This study set out to investigate the effect of bio-fertilizers containing nitrogen-fixing bacteria and phosphorus on grain yield and yield components of Faba bean. To this end a factorial experiment on the basis of randomized complete block design with four replications was conducted in Behbahan region at South eastern part of Khuzestan Province of Iran. The experimental treatments included Nitroxin containing nitrogen-fixing bacteria in three levels of 0, 250, and 500 cc per 1000 m<sup>2</sup> and phosphorus bio-fertilizer (fertile 2 phosphates) containing phosphorus-fixing bacteria in three levels of 0, 100, and 150 g.ha<sup>-1</sup>. The results showed that the effect of treatments with phosphorus bio-fertilizer and Nitroxin on grain yield, biological yield, and harvest index, number of pods per plant, number of grain per pod, 1000-grain weight, and leaf area index was significant at 1% level. The highest grain yield, biological yield, harvest index, number of pods per plant, number of grains per pod, 1000-grain weight, and leaf area index belonged to the treatment with consumption of 500 cc Nitroxin per 1000 m<sup>2</sup> and 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer and the lowest rates belonged to the control treatment.

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**Keywords:** Bio-fertilizer, Grain yield, Nitroxin, *Vicia faba*.

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### INTRODUCTION

Faba bean is an important feeding crop grown in winter season of south west of Iran (Morteza *et al.*, 2013). The seeds not only provide a cheap source of protein but also a food of high calorific and nutritive value especially in the diet of low income people (Rahi, 2013). Consumption and overuse of chemical inputs in conventional farming over the last few decades have caused many en-

vironmental problems such as contamination of water and the soil resources, reduction of food quality, disrupting biological balance in soil that cause irreparable damage to ecosystems (Soudra *et al.*, 2002). Many attempts took place in the recent years in order to avoid the harmful effects of chemicals and pesticides (Fedotova *et al.*, 2009).

Bio-organic farming systems are the recent trend, newly introduced, in the agricultural practice for getting clean products (Sharaf *et al.*, 2009). Application of these systems in the agriculture regimes became inevitable to put an end to the unwise doses of harmful chemicals used for increasing productivity of different crops or controlling the deleterious insects that hide enormous amounts of metals and hydrocarbon compounds negatively affect the surrounding environment in which human begins, animals and plants are living (Gomaa and Attia, 1998). Obviously, combined application of organic fertilizer and urea fertilizer or combination urea fertilizer and polyamines significantly increased yield, vegetative growth and chlorophyll index (Oad *et al.*, 2004, Zeid, 2008). The basic solution to such problems is moving towards sustainable agriculture based on the optimal use of elements within the field such as bio-fertilizers (Mekail *et al.*, 2005). Biological fertilizers such as Nitroxin contain a series of fixing bacteria which contains a set of nitrogen-fixing bacteria of *Azotobacter* and *Azospirillum* genus (Afageh *et al.*, 2006). Nitrogen fertilizers replacement through biological nitrogen fixation leads to the reduction of environmental issues resulting from the leaching of soil nitrate nitrogen. *Azotobacter* inoculation increases yield of different plants about 10 to 15% in average (Idris, 2003, Kandil *et al.*, 2013). Phosphorus is a major nutrient, especially for legumes. It is considered the second essential nutrient element for both plants and microorganisms (Ahmed *et al.*, 2007). In spite of the considerable addition of phosphorus to soil, the amount available for plant is usually low. Phosphate dissolving bacteria and soil microorganisms can play an important role in improving plant growth and phosphate up-

take efficiency by releasing phosphorus from rock or tri-calcium phosphate. Many researchers showed positive effect of phosphorus fertilization on faba bean (El-Gizawy and Mehasen, 2009). Kazemi *et al.* (2005) investigated the effect of seeds inoculation with growth promoting bacteria on yield and yield components of two soybean cultivars and reported that inoculation significantly increased number of pod per plant, number of grain per plant, 1000-grain weight and grain yield of soybean. Consumption of phosphate fertilizers in soil supplies the need of crops to phosphorus. However, the problem of such fertilizers is their fixation with soil complex which makes about two-thirds of them unusable for plants (Yilmaz, 2008). By using phosphate solubilizing microorganisms in crop root zone and soil, the capability of phosphate availability through insoluble phosphate resources will increase and the efficiency of phosphate fertilizers such as superphosphate will improve (Gad *et al.*, 2012). Biological phosphorus fertilizers are good substitutes for phosphate chemical fertilizers. They contain two phosphate solubilizing bacteria which dissolve the soil phosphorus using two mechanisms of organic acid and phosphatase acid secretion (Fedotova *et al.*, 2009). Hamed (2003) showed that consumption of phosphate bio-fertilizer in maize, soybean, and wheat revealed that phosphate biological fertilizer significantly increased the yield. Jadhav *et al.* (1998) observed increasing to 68.4 percent dry weight of corn plants by using phosphate solubilizing bacteria. Abd-Alrahman Rehab *et al.* (2002) stated that use of phosphorus bio-fertilizer in wheat, compared with chemical fertilizers, led to reduction of chemical fertilizer consumption, increase of product, uniformity of the farm, thickness of stems, increase of spikes length, plant

resistance to diseases and frost, increase of tillering and ultimately decrease of environmental damage. 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. The highest values of vegetative growth, oil yield, chlorophyll content and N.P.K. percentages were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer (Ahmed *et al.*, 2013). Inoculation of *Pseudomonas striata* (P.S.B.) + V.A.M. fungi + *Azotobacter* recorded significantly higher chlorophyll content in leaf, as compared to PSB + *Azotobacter* and V.A.M + *Azotobacter* inoculation (Saleh *et al.*, 2015). The present research was carried out to investigate the effect of application of bio-fertilizers containing nitrogen- and phosphorus fixing bacteria on some quantitative parameters of faba bean.

## MATERIALS AND METHODS

### *Field and treatment information*

The research was conducted in 2013 in a field located in Behbahan region, in the Southeastern part of Khuzestan Province of Iran at latitude 30° 36' N, and longitude 50° 14' E and 313 m above the sea level. The factorial experiment was

carried out with using randomized complete block design at four replications. Experimental treatments included Nitroxin bio-fertilizer containing nitrogen-fixing bacteria in three levels of 0, 250, and 500 cc per 1000 m<sup>2</sup> and phosphorus bio-fertilizer containing phosphorus-fixing bacteria in three levels of 0, 100, and 150 g. ha<sup>-1</sup> which was prepared as seeding.

### *Crop Management*

Each plot consisted of four planting lines as long as 6 m, and the distance between the rows was 50 cm. The distance between plots was 50 cm. In order to determine the soil properties and its physical and chemical properties, samples were taken from the farm (Table 1). 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, harvest operation was carried out.

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

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

### *Traits measure*

The studied traits included grain yield, biological yield, and harvest index, number of pods per plant, number of grain per pod, 1000-grain weight and leaf area index. In order to determine grain yield 1 m<sup>2</sup> was and the grain yield was calculated in kg.ha<sup>-1</sup>. In order to

measure yield components such as number of pods per plant and the number of grain per pod ten plants were randomly selected from each experimental plot after physiological investigation and were evaluated and the means were calculated for each trait. Moreover, in order to measure 1000-

grain weight, five 200-grain samples were counted and the weight was measured. Harvest index was calculated by formula as follow (FAO, 2002):

**Formula 1.** Harvest Index (HI) = Grain Yield (G.Y)/Biological Yield (B.Y) 100.

To measure leaf area changes the samples were taken every 10 days. Five samples were taken and in each sampling stage, five plants were taken and leaf area was measured. Leaf area index was calculated using disk method.

#### *Statistical analysis*

All data analysis was done using SAS software (Ver. 8) and the means were compared using Duncan's multi range test at 1% probability level.

## **RESULTS AND DISCUSSION**

### **Biological Yield**

The ANOVA results showed that the effect of Nitroxin, fertile 2 phosphate and their interactive effect on biological yield were significant at 1% probability level (Table 2). Mean comparison results showed that in Nitroxin treatments the highest biological yield belonged to 500 ml Nitroxin and the lowest one belonged to control treatment (Table 3). The increase of biological yield by Nitroxin can be attributed to the activity of fertilizer microorganisms which produced hormones especially gibberellin led to the increase of stem foliage and shoots. Microorganisms producing this hormone are able to stimulate their host plants growth through the synthesis of released active GAS (Said, 1998). In phosphorus bio-fertilizer treatments, the highest biological yield by belonged to 150 g.ha<sup>-1</sup> fertile 2 phosphate and the lowest one belonged to the control treatment. According to the results it

can be stated that the application of biological phosphorus up to 100 g significantly increased biological yield but after that they were statistically in the same level (Table 3). The results of the interactive effect of Nitroxin and phosphorus bio-fertilizer showed that the highest biological yield belonged to the treatment with application of 500 cc per 1000 m<sup>2</sup> Nitroxin and 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer. The lowest biological yield belonged to the control treatment (Table 4). Apparently, the concurrent use of Nitroxin and phosphorus bio-fertilizer could increase biological yield in faba bean due to having sufficient nutrients and appropriate growth regulators and also due to the activity of microorganisms. El-Masri *et al.* (2002) studied the effect of fertile 2 phosphate on faba bean and showed that as the rate of phosphorus bio-fertilizers increased, the foliage and biological yield of faba bean significantly increased. The research conducted by Morteza *et al.* (2013) confirmed the significant improvement of biological yield due to the use of phosphate solubilizing bacteria in an herb from Euphorbia family.

### **Grain Yield**

results showed that the effect of Nitroxin, phosphorus bio-fertilizer and their interactive effect on grain yield were significant at 1% probability level (Table 2). Mean comparison results showed that in Nitroxin treatments highest grain yield belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin which was not significantly different from 250 cc per 1000 m<sup>2</sup> Nitroxin and lowest grain yield belonged to control treatment (Table 3).

**Table 2.** The ANOVA results of nitroxin and fertile 2 phosphates on measured traits

S.O.V	df	Biological yield	Grain yield	Harvest index	Number of pod per plant	Number of grain per pod	1000-grain weight	Leaf area index
Replication	3	1986945*	3058*	2.08*	0.03*	0.25*	419*	0.001*
Nitroxin (N)	2	7034007**	4886711**	147**	53.9**	5.1**	241008**	5.2**
Fertile 2 Phosphate (P)	2	2367834**	3248233**	152**	35.8**	3.7**	78508**	1.16**
Nitroxin × Fertile 2 phosphate	4	634235**	700965**	34**	1.03 <sup>ns</sup>	0.25 <sup>ns</sup>	9020**	0.049**
Error	24	45893	550	0.59	0.57	0.09	40.5	0.007
CV (%)	-	7.7	6.2	6.7	5.4	8.6	5.1	5.2

\*\* and \*: Significant at 1% and 5% probability level, respectively. ns: not significant.

**Table 3.** Mean comparison of nitroxin and fertile 2 phosphates on measured traits

Treatment	Biological yield (kg.ha <sup>-1</sup> )	Grain yield (kg.ha <sup>-1</sup> )	Harvest index (%)	Number of pod per plant	Number of grain per pod	1000-grain weight (g.ha <sup>-1</sup> )	Leaf area index
<b>Nitroxin fertilizer (g.ha<sup>-1</sup>)</b>							
N0= 0	6859 <sup>b</sup>	2953 <sup>c</sup>	43.05 <sup>b</sup>	11.6 <sup>c</sup>	3.6 <sup>c</sup>	1066 <sup>b</sup>	2.9 <sup>b</sup>
N1= 250	7694 <sup>a</sup>	3911 <sup>b</sup>	50.83 <sup>a</sup>	13.8 <sup>b</sup>	3.7 <sup>b</sup>	1326 <sup>a</sup>	4.0 <sup>a</sup>
N2=500	8626 <sup>a</sup>	4402 <sup>a</sup>	51.03 <sup>a</sup>	16.5 <sup>a</sup>	4.1 <sup>a</sup>	1368 <sup>a</sup>	4.3 <sup>a</sup>
<b>Fertile 2 phosphate (g.ha<sup>-1</sup>)</b>							
P0= 0	7136 <sup>b</sup>	3068 <sup>b</sup>	42.99 <sup>b</sup>	11.7 <sup>c</sup>	2.7 <sup>b</sup>	1147 <sup>b</sup>	3.3 <sup>b</sup>
P1= 100	7978 <sup>a</sup>	4021 <sup>a</sup>	50.40 <sup>a</sup>	14.7 <sup>b</sup>	3.8 <sup>a</sup>	1294 <sup>a</sup>	3.9 <sup>a</sup>
P2= 150	8065 <sup>a</sup>	4178 <sup>a</sup>	51.80 <sup>a</sup>	15.5 <sup>a</sup>	3.8 <sup>a</sup>	1320 <sup>a</sup>	4.0 <sup>a</sup>

According to Duncan's multi range test the means with similar letters are not significantly different from each other at 5% probability level.

**Table 4.** Mean comparison of interaction effects of nitroxin and fertile 2 phosphate on measured traits

Treatment	Biological yield (kg.ha <sup>-1</sup> )	Grain yield (kg.ha <sup>-1</sup> )	Harvest index (%)	Number of pod per plant	Number of grain per pod	1000-grain weight (g.ha <sup>-1</sup> )	Leaf area index
N0P0	6720 <sup>d</sup>	2585 <sup>e</sup>	38.3 <sup>d</sup>	13.8 <sup>a</sup>	3.8 <sup>a</sup>	1032 <sup>e</sup>	2.3 <sup>e</sup>
N0P1	6848 <sup>d</sup>	3020 <sup>d</sup>	42.0 <sup>c</sup>	13.7 <sup>a</sup>	4.0 <sup>a</sup>	1071 <sup>e</sup>	3.0 <sup>d</sup>
N0P2	7010 <sup>cd</sup>	3256 <sup>cd</sup>	44.2 <sup>bc</sup>	14.0 <sup>a</sup>	4.0 <sup>a</sup>	1095 <sup>e</sup>	3.3 <sup>d</sup>
N1P0	7236 <sup>c</sup>	3546 <sup>c</sup>	46.8 <sup>b</sup>	13.8 <sup>a</sup>	4.0 <sup>a</sup>	1188 <sup>d</sup>	3.7 <sup>c</sup>
N1P1	7891 <sup>b</sup>	4006 <sup>b</sup>	48.7 <sup>ab</sup>	13.7 <sup>a</sup>	4.0 <sup>a</sup>	1378 <sup>b</sup>	4.1 <sup>ab</sup>
N1P2	7955 <sup>b</sup>	4181 <sup>b</sup>	50.4 <sup>a</sup>	14.0 <sup>a</sup>	4.0 <sup>a</sup>	1411 <sup>a</sup>	4.2 <sup>ab</sup>
N2P0	7453 <sup>bc</sup>	3073 <sup>d</sup>	39.4 <sup>d</sup>	14.0 <sup>a</sup>	3.8 <sup>a</sup>	1220 <sup>c</sup>	4.0 <sup>b</sup>
N2P1	9196 <sup>a</sup>	5038 <sup>a</sup>	52.8 <sup>a</sup>	14.0 <sup>a</sup>	3.8 <sup>a</sup>	1433 <sup>a</sup>	4.5 <sup>a</sup>
N2P2	9230 <sup>a</sup>	5096 <sup>a</sup>	53.2 <sup>a</sup>	13.8 <sup>a</sup>	4.0 <sup>a</sup>	1453 <sup>a</sup>	4.5 <sup>a</sup>

According to Duncan's multi range test the means with similar letters are not significantly different from each other at 5% probability level.

The increase of grain yield by *Azotobacter* can be related to the growth prompting substances which are produced by microbes in addition to fixed nitrogen. Moreover, it seems like that the highest effect of nitrogen on grain yield is due to the increase of number of pod per plant by preventing the abortion of young flowers and pods (El-Habbasha *et al.*, 2007). In phosphorus bio-fertilizer treatments, the highest grain yield belonged to 150 g.ha<sup>-1</sup> fertile 2 phosphate that was not significantly different from 100 g.ha<sup>-1</sup>, and the lowest grain yield belonged to control (Table 3). El-Gizawy and Mehasen (2009) concluded that application of phosphate solubilizing bacteria increased yield and yield components of soybean comparison with chemical fertilizers. The results of the interactive effect of Nitroxin and phosphorus bio-fertilizer showed that the highest grain yield belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin and 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer which was not significantly different from the treatment with application of 500 cc Nitroxin and 100 g.ha<sup>-1</sup> phosphorus bio-fertilizer. (Table 4). Afageh *et al.* (2006) showed that phosphorus fertilizer by itself had no significant effect on wheat grain yield, but biological phosphate fertilizer (fertile 2), both by itself and in combination with other fertilizers had a significant effect on yield. Said (1998) reported that use of bio-fertilizers increased faba bean efficiency.

### Harvest Index

The effect of Nitroxin, phosphorus bio-fertilizer and their interactive effect on harvest index were significant at 1% probability level (Table 2). Mean comparison results showed that in Nitroxin treatments the highest harvest index belonged to 500 ml Nitroxin which was not significantly different from the

treatment with application of 250 ml Nitroxin and the lowest one belonged to control. In phosphorus bio-fertilizer treatments, the highest harvest index belonged to 150 g.ha<sup>-1</sup> fertile 2 phosphate which was not significantly different from 100 g.ha<sup>-1</sup> (Table 3). The results of the interactive effect of Nitroxin and phosphorus bio-fertilizer showed that the highest harvest index belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin and 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer which was not significantly different from the treatment with application of 500 cc per 1000 m<sup>2</sup> Nitroxin and 100 g.ha<sup>-1</sup> phosphorus bio-fertilizer (Table 4). Mekail *et al.* (2005) reported that application of bio-fertilizers not only increased crop yield and yield components but also decreased the consumption of chemical fertilizers.

### Number of pod per plant

Number of pods per plant is the most variable trait among the yield components. The ANOVA results showed that the effect of Nitroxin and phosphorus bio-fertilizer on number of pods per plant was significant at 1% probability level, but the interactive effect of Nitroxin and phosphorus bio-fertilizer on this trait was not significant (Table 2). Mean comparison results showed that in Nitroxin treatments the highest number of pods per plant belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin and the lowest one belonged to control (Table 3). The positive effects of application of bio-fertilizers can be attributed to the increase of water and nutrient absorption due to the development of roots and also the biological process of nitrogen. In both treatments of Nitroxin and phosphorus bio-fertilizer as the fertilizers increased the number of pods per plant increased too, so that the highest number of pods per plant was observed at the highest levels of applied treat-

ments. The interactive effects of Nitroxin and phosphorus bio-fertilizer on number of pod per plant was not significant, i.e. treatments had similar reactions to simultaneous application of Nitroxin and phosphorus bio-fertilizer in relation to the number of pods per plant (Table 4). The research conducted by Hamed (2003) showed that increasing of nitrogen application or its absorption increased number of pods per plant. Afageh *et al.* (2006) also confirmed the results of the research.

#### Number of grain per pod

The ANOVA results showed that the effect of Nitroxin and phosphorus bio-fertilizer on the number of grains per pod was significant at 1% level, but their interactive effect on this trait was not significant (Table 2). Number of grain per pod is mainly affected by genetic and is the most stable yield component in legumes (Hamed, 2003). Mean comparison results showed that in Nitroxin treatments the highest number of grains per pod belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin and the lowest one belonged to control treatment. Zeid (2008) stated that in their research Azotobacter by itself could not make a significant difference in number of grain per pod and adding N.P.K. fertilizers is necessary. In phosphorus bio-fertilizer treatments, the highest number of grain per pod belonged to 100 and 150 g.ha<sup>-1</sup> fertile 2 phosphate and the lowest number of grains per pod belonged to control (Table 3). The interactive effect of Nitroxin and phosphorus bio-fertilizer on number of grains per pod was not significant (Table 4).

#### 1000-Grain Weight

The results showed that the effect of Nitroxin, phosphorus bio-fertilizer and their interactive effect on 1000-grain weight were significant at 1% probab-

ity level (Table 2). Nitroxin treatment increased 1000-grain weight as the rate of Nitroxin consumption increased, therefore the highest 1000-grain weight belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin (Table 3). Biari *et al.*, (2007) reported that inoculation of maize with Nitroxin positively and significantly affected the weight of grain per plant, total plant weight, and grain yield compared with control treatment. In phosphorus bio-fertilizer treatments, the highest weight of 1000-grain belonged to 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer which was not significantly different from the treatment with application of 100 g phosphorus bio-fertilizer (Table 3). The results of the interactive effect of Nitroxin and phosphorus bio-fertilizer showed that the highest weight of 1000-grain belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin and 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer which was not significantly different from the treatment with application of 500 cc per 1000 m<sup>2</sup> Nitroxin and 100 g.ha<sup>-1</sup> phosphorus bio-fertilizer. Lowest weight of 1000-grain belonged to control and phosphorus bio-fertilizer (Table 4).

#### Leaf Area Index

The effect of Nitroxin, phosphorus bio-fertilizer and their interactive effect on leaf area index were significant at 1% probability level (Table 2). Mean comparison results showed that in Nitroxin treatments the highest leaf area index belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin that was not significantly different from 250 cc per 1000 m<sup>2</sup> Nitroxin (Table 3).

In phosphorus bio-fertilizer treatments, the highest leaf area index belonged to 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer which was not significantly different from the treatment with application of 100 g.ha<sup>-1</sup> phosphorus bio-

fertilizer (Table 3). The results of the interactive effect of Nitroxin and phosphorus bio-fertilizer showed that the highest leaf area index belonged to 500 cc per 1000 m<sup>2</sup> Nitroxin and 150 g.ha<sup>-1</sup> phosphorus bio-fertilizer which was not significantly different from the treatment with application of 500 cc per 1000 m<sup>2</sup> Nitroxin and 100 g.ha<sup>-1</sup> phosphorus bio-fertilizer. The lowest leaf area index belonged to control and phosphorus bio-fertilizer (Table 4). Bio-fertilizers can increase leaf area index due to sufficient nutrients, capability of nutrients uptake and producing growth regulating substances (Ratti *et al.*, 2001).

#### CONCLUSION

According to results of the experiment the best treatment in grain yield, biological yield, 1000-grain weight, harvest index, and leaf area index was 500 ml Nitroxin per 1000 m<sup>2</sup> and 150 g phosphorus bio-fertilizer.

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