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ORIGINAL ARTICLE

Changes in yield and yield components of soybean (*glycine max* L.) under application of Phosphate and Nitrogen Bio-fertilizers

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ABSTRACT
A field experiment was conducted to investigate the effect of a some N and P biofertilizars and symbiotic nitrogen fixing bacterium Bradyrhizobium japonicum on growth, yield and yield components of soybean (Glycine max (L.) Williams) in Islamic Azad University, Boroujerd branch, Iran at 2013. The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were Nitrogen bio-fertilizers in three levels (a_1=Rhizobium japanicum, a_2= Nitroxin, a_3= Nitrokara) and phosphor biofertilizer in three levels (b_1= Agronomy special Phosphate Barvar$_2$, b_2= Soybean special Phosphate Barvar2, b_3= Biosuperphosphate). Results showed that effect of N and P bio-fertilizers and interaction effect of N and P bio fertilizers on all treats in this study were significant. Based on the results, combined application of Bradyrhizobium japonicum bacteria and Soybean special Phosphate Barvar$_2$ had the highest plant height. Non application of each N and P biofartilizers has a lowest pod length and application of any biofertilizers had high pod length. Single application of Agronomy special Phosphate Barvar$_2$ (P biofertilizer) in non-application of N biofertilizer treatments had the highest number of pod per plant and 1000 grain weight. However, results showed that, combined application of Nitroxin with Soybean special Phosphate Barvar2 had a highest biomass and grain yield. Therefore, combined application of these fertilizers had more efficiency because of some positive interaction between their microorganisms result to synergistic effect and increase in yield components and in final grain yield. Then we can propose this biofertilizer combination to high yield in soybean for farmers of Kangavar region.
Key words: Bio-fertilizer, Soybean and Yield components

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INTRODUCTION

Soybean (*Glycine max* L.) is one of the most widely adopted grain legumes in the world. Soybean is used in the production of oil and protein. World area soybean cultivation was approximately 79,410,495 acres in 2010. In general, the areas under cultivation has increased from 1991 onwards 23.5 percent in Asia, North America 42.3 %, Latin America 31%, Europe 8.1 %, 2.1, Africa 2% and Oceania Africa less than 0.1 % of the world's cultivated area has been allocated [10]. Rainfed crops grown in semiarid regions experience unpredictable water deficits during their life cycle [21]. Nodulation of soybean requires specific *Bradyrhizobium* species [1]. In soils where the soybean crop has not been grown previously, compatible populations of *bradyrhizobia* are seldom available [1]. The nitrogen demand of soybean can be supplied via biological nitrogen fixation through the inoculation with selected *Bradyrhizobium japonicum*/B.elkanii strains [19].Fertilizer management is the most important factors in successful agriculture affecting yield, it components, quality and quantity [26]. Mineral nutrient is one of the key factors limiting agricultural productivity in the arid to semiarid areas [28]. In the present century positive safe effect of biofertilizers on growth, yield and yield component of many crops was reviled. Conventional farming methods succeed in today's world is not acceptable to use the resource management and relying too much on synthetic inputs such as fertilizers and chemical pesticides can cause auxiliary power injector and the ecosystem of unstable farming [17, 20]. The use of bio-fertilizers and plant growth enhancer, particularly bacteria, is most important food plants in integrated pest management strategies for sustainable agriculture systems in combination with adequate inputs of chemical and bio fertilizers [24]. Including bio-fertilizers containing numerous microorganisms can named Azotobacter, Nitroxin, Tiobacilus, mycorrhiza and Biosulphure [6] and mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms [11]. In contrast with these symbioses, some diazotrophs, such as *Azospirillum* spp., *Azoarcus* spp. and Herbaspirillum, form associative and/or endophytic relationships with a wide variety of plant roots including those of cereals. In all these associations and symbioses, for the host plants the expected benefit of the interaction is the fixed nitrogen provided by the symbiotic partner, which, in return, receives reduced carbon and possibly all the other nutrients it requires. In addition, the symbiotic or endophytic plant structure colonized by the nitrogen-fixing microorganisms may provide the appropriate conditions for protecting the nitrogenase complex from oxygen exposure [22].

Biofertilizers supply nutrient for plant needs, minimizes leaching, and therefore improves fertilizer use efficiency [25]. Application of Supernitroplassbiofertilizer with Phosphate barvar2 treatment has the highest seed yield (7.6 ton/ha) and non-application of biofertilizers treatment has the Pishtaz cultivar has the lowest seed yield (6.3 ton/ha) [3]. They told that for gave the highest seed yield we should apply both nitrogen and phosphate biofertilizers. In maize application nitrogen and phosphate biofertilizers increased yield and yield components of maize under Boroujerd environmental condition [5]. They suggested that effect of nitrogen and phosphate biofertilizers were evaluated positively, there were an increase in plant height, ear weight, and number of grain per cob, grain yield and biomass yield. Also in another research application of nitrogen and phosphate biofertilizers increased yield and yield components of barley under Boroujerd environmental condition [4]. They suggested that grain yield and biomass yield increasing was reported with the biofertilizer application 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. Increasing yield was attributed to the plant growth promoting substances by root colonizing bacteria more than the biological nitrogen fixation, [12] stated that yield increased due to promoting root growth which in turn enhancing nutrients and water uptake from the soil.

Therefore this study was laid out for evaluation of effects of N and P Bio fertilizers on yield and yield components of soybean.

MATERIALS AND MTHODS

Field material and Experimental design

This study was carried out in order to evaluate the effects of nitrogen and phosphate bio fertilizers on yield components of soybean (Williams cultivar) in the faculty of agronomy and plant breeding, Islamic Azad University, Boroujerd Branch (experiment station: Kangavar), Iran during the growing seasons 2012- 2013. The experimental region has a continental semi-arid climate with annual precipitation of 327 mm. Soil property of experimental field showed in table1.

Table 1. Soil property of experimental location.

Soil Texture	Sand (%)	Silt (%)	Clay (%)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	K (mg/kg)	P (mg/kg)	N (%)	pH	EC (mmohs/cm)	Depth
L	29	45	26	0.48	0.54	1.26	1.38	232	13.1	0.2	8.09	0.4	0-30

Treatments

The experiment was laid out in a split-plot design based on randomized block design with three replications. Treatments were Nitrogen bio-fertilizers in three levels (a_1=Rhizobium japanicum, a_2= Nitroxin, a_3= Nitrokara) and phosphor biofertilizer in three levels (b_1= Agronomy special Phosphate Barvar$_2$, b2= Soybean special Phosphate Barvar2, b_3= Biosuperphosphate) with control for them.

Yield and yield components determination

In this field experiment there were 6 rows in each from 16 plot; rows were 6 m long with 0.5 m row spacing and plant to plant spacing was 10 cm too. At maturity, two outer rows for each plot, 50 cm from each end of the plots, were left as borders and the middle one m^2 of the four central rows were harvested. Then yield components were calculated as standard methods with using 8 plant. To determine grain yield and biomass yield, we removed and cleaned all the seeds produced within middle one m^2 of the four central rows in each plot. Then grain yield and biomass yield recorded on a dry weight basis. Yield was defined in terms of grams per square meter and quintals per hectare. Replicated samples of clean seed (broken grain and foreign material removed) were sampled randomly and 1000-grain were counted and weighed.

Statistical analysis

The statistical analyses to determine the individual and interactive effects of time cultivation and weeds control methods were conducted using JMP 5.0.1.2 (SAS Institute Inc., 2002). Statistical significance was declared at P≤0.05 and P≤0.01. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis (23).

RESULTS

Plant height: for soybean ANOVA table showed that, the effect of N and P bio-fertilizers and interaction between them on plant height were significant at 1% (table 2). The comparison of the mean values for interaction between N and P bio-fertilizers on plant height showed that combined application of *Rhizobium japanicum* bacteria and Soybean special Phosphate Barvar$_2$ had the highest (140 cm) and the control treatment had the lowest plant height (112) and difference between them was significant (figure 1).

Table 2. Analysis of variance (mean squares) for yield and yield components of soybean under application of N and P bio-fertilizer

	df	Plant height	Pod length	N number of pod	1000 grain weight	Biological yield	Grain yield
Replication	2	3.33	0.18	22.03	26.27	1002192.2	76231.19
N biofertilizer	3	389.85**	0.33**	502.53	406.39**	5586257**	1939865.84**
Error (a)	6	7.83	0.02	6.57	49.41	331317.2	135960.9
Pbiofertilizer	3	91.92**	0.22**	246.16**	586.67**	13024218.2**	3503652.5**
N*P	9	144.6**	0.17**	232.73**	624.69**	25389082.5**	2580570.02**
Error (b)	24	7.17	0.04	7.88	29.75	233343.3	41319.09
CV (%)		2.08	4.61	6.1	3.72	5.11	5.07

ns: Non-significant, * and **:Significant at 5 and 1% probability levels, respectively.

Pod length: The effect of N and P bio-fertilizers and interaction between them on pod length were significant at 1% (table 2). The comparison of the mean values of the pod length for interaction effect of N and P bio-fertilizers showed that application of any N and P biofertilizers has a positive effect on it. In all combined treatment pods has a high (4.2-4.6 cm) length rather than control treatment. Non application of each N and P biofertilizers has a lowest (3.8 cm) pod length and difference of it with other treatment was significant (figure 2).

Number of pod per plant: The results of analysis of variance showed that, the effect of N and P bio-fertilizers and interaction between them on number of pod per plant were significant at 1% (table 2). The comparison of the mean values for interaction of N and P bio-fertilizers on number of pod per plant for soybean showed that single application of Agronomy special Phosphate Barvar$_2$ (P biofertilizer) in non-application of N biofertilizer treatments had the highest (78) and the combined application of *Rhizobium japanicum* and Agronomy special Phosphate Barvar treatment had the lowest number of pod per plant [38] and difference between them was significant (figure 3).

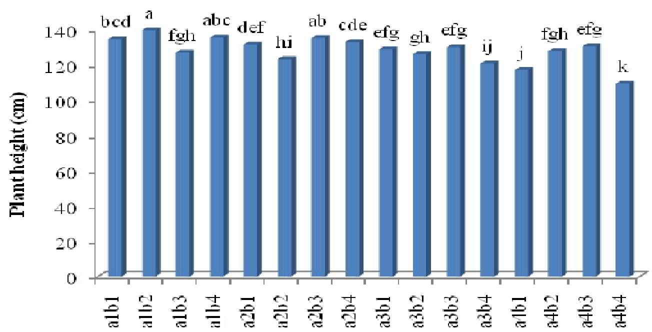

Figure 1.Interaction effect of N and P bio-fertilizers on plant height in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).

(a1=Rhizobium japanicum, a2= Nitroxin, a3= Nitrokara, a4= Control, b1= Agronomy special Phosphate Barvar2 , b2= Soybean special Phosphate Barvar2, a3= Biosuperphosphate, b4= Control)

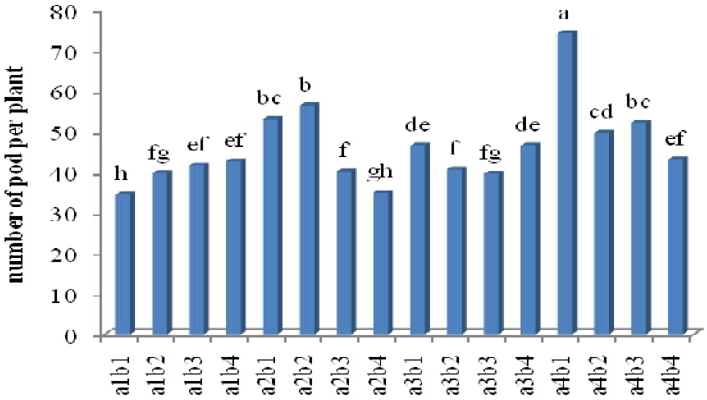

Figure 2.Interaction effect of N and P bio-fertilizers on number of pod per plant in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(a1=Rhizobium japanicum, a2= Nitroxin, a3= Nitrokara, a4= Control, b1= Agronomy special Phosphate Barvar2 , b2= Soybean special Phosphate Barvar2, a3= Biosuperphosphate, b4= Control)

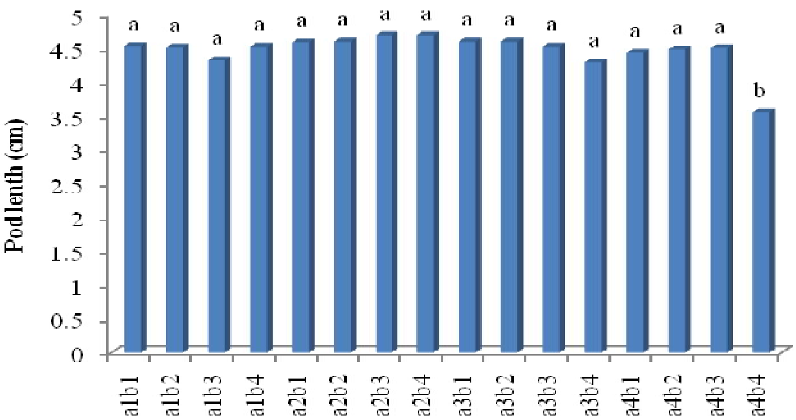

Figure 3.Interaction effect of N and P bio-fertilizers on pod length in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(a1=Rhizobium japanicum, a2= Nitroxin, a3= Nitrokara, a4= Control, b1= Agronomy special Phosphate Barvar2 , b2= Soybean special Phosphate Barvar2, a3= Biosuperphosphate, b4= Control)

1000 grain weight: The results showed that, the effect of N and P bio-fertilizers and interaction between them on 1000 grain weight were significant at 1% (table 2). The interaction effects of comparison values for 1000 grain weight showed that single application of Agronomy special Phosphate Barvar$_2$ (P biofertilizer) in non-application of N biofertilizer treatments had the highest (179 g) and the control treatment in all N and P biofertilizer levels had the lowest 1000 grain weight (110 g) and differences between them were significant (figure 4).

Grain yield: The results showed that, effect of N and P bio-fertilizers and interaction between them on grain yield were significant at 1% (table 2). The comparison means values for grain yield showed that combined application of Nitroxin with Soybean special Phosphate Barvar2 had a highest (6200 kg/ha) and the control treatment in all N and P biofertilizer levels had the lowest grain yield (2000 kg/ha) and difference between them were significant (figure 5).

Biomass yield: The effect of N and P bio-fertilizers and interaction between them on biomass yield were significant at 1% (table 2). The comparison of the mean values of interaction effect of N and P biofertilizers for biomass yield showed that combined application of Nitroxin with Soybean special Phosphate Barvar2 had a highest (13800 kg/ha) and the control treatment in all N and P biofertilizer levels had the lowest biomass yield (4000 kg/ha) and difference between them were significant (figure 6).

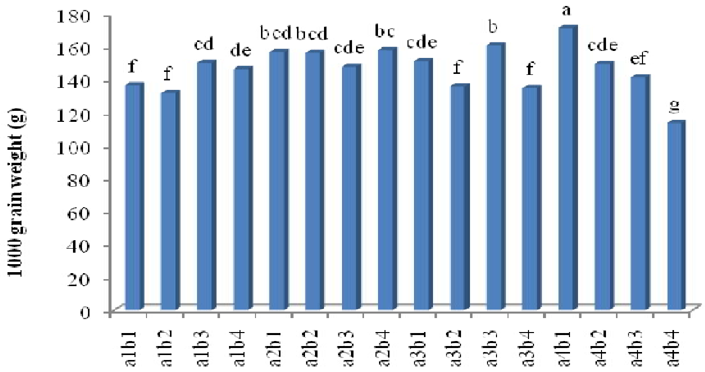

Figure 4.Interaction effect of N and P bio-fertilizers on 1000 grain weight in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(a1=Rhizobium japanicum, a2= Nitroxin, a3= Nitrokara, a4= Control, b1= Agronomy special Phosphate Barvar2 , b2= Soybean special Phosphate Barvar2, a3= Biosuperphosphate, b4= Control)

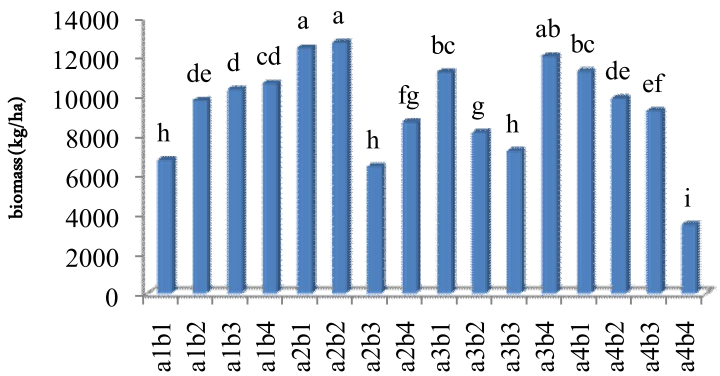

Figure 5.Interaction effect of N and P bio-fertilizers on biomass yield in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(a1=Rhizobium japanicum, a2= Nitroxin, a3= Nitrokara, a4= Control, b1= Agronomy special Phosphate Barvar2 , b2= Soybean special Phosphate Barvar2, a3= Biosuperphosphate, b4= Control)

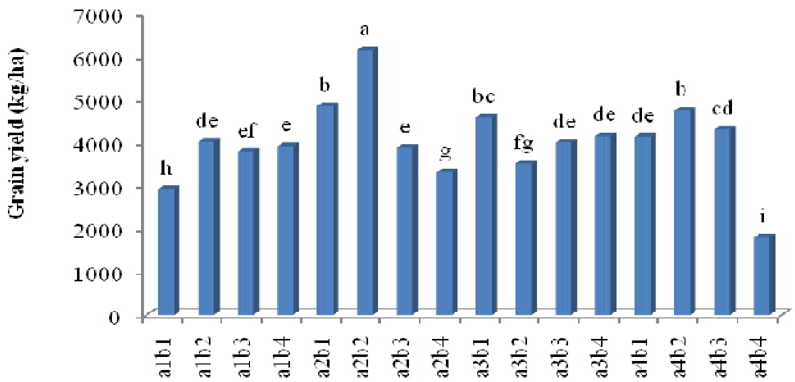

Figure 6. Interaction effect of N and P bio-fertilizers on grain yield in soybean.
Means by the uncommon letter in each column are significantly different (p<0.05).
(a1=Rhizobium japanicum, a2= Nitroxin, a3= Nitrokara, a4= Control, b1= Agronomy special Phosphate Barvar2 , b2= Soybean special Phosphate Barvar2, a3= Biosuperphosphate, b4= Control)

DISCUSSION

The results showed that the various bio fertilizer treatments and specially interaction between them had very significant effect on yield and yield components of soybean (Table 2). For plant height it was observed that treatment of *rhizobium japanicum* × soybean special phosphate barvar2 treatment was 27.2 % more than control (figure 1). This might be the result of the microorganisms involved in P solubilization, which can enhance plant growth by increasing the efficiency of biological fixation, enhancing the availability of trace elements and by the production of plant growth promoting substances [12]. Darzi et al, (2008) showed that biophosphate biofertilizer had a significant effects on height of fennel (9). Height of wheat was increased significantly in inoculation with *P. cepacia R85*, R55, P. aeuroginosa R80, R61, P. putida R104 and P. fluorescens R22 bacterial biofertilizers significantly compared to control [2]. Increase in plant growth, nodulation and yield of soybean by B. *japonicum* strains have also been reported in other countries like Canada [15], South Africa [7].

From the data presented (figure 3), it seems that application of any of biofertilizers as single or combined increased pod length as well and non-application of any of this biofertilizers had minimum pod length in all treatments. A good supply of organic manure could result more productive tillers, more number of spikes per unit area, number of grains per spike and grain yield (27). For 1000 grain weight agronomy special Phosphate Barvar2 had 50.74% percent increase compared to the control. Non application of bifertilizers had lowest 1000 grain weight. Kazemi et al. [14] reported that soybean seed inoculation with *rhizobial* bacteria significantly increased seed thousand weigh[. Zhang et al (2002) reported that inoculation with B. *japonicum* bacteria increased 100 seed weight of two soybean cultivars (27).

In this study application of combined of any different bio fertilizers was not positive for number of pod per plant. The number of pods per plant ranged from 78 pods per plant in plants fertilized with N and P chemical fertilizers to 38 pods per plant in control. Highest number of pod per plant achieved in application of Agronomy special Phosphate Barvar$_2$ P biofertilizer and interaction effect of these biofertilizers on this treat was antagonistic. Nabila et al. (2007) observed that application of *Azospirillum* as single on wheat had significant effect on number of grain per spikelet (18).

For biomass yield application of Nitroxin with Soybean special Phosphate Barvar2 had a highest biomass yield (figure 5). This can be caused by stimulating secretion of growth hormones which is produced by this bacteria and their effect on plant growth. Non application of biofertilizers treatment had a lowest biomass yield. Kandil et al. [13] reported that the use of biological fertilizers in sugar beet, significantly increased plant dry weight.

In the present study combined application of Nitroxin with soybean special Phosphate Barvar2 increased grain yield 42% compared to control. Mahfouz and Sharaf-Eldin [16] reported that phosphorous solvent bacteria have the ability to produce organic acids that would increase solubility of phosphorus available for plants. Continuous and stable supply of mineral elements especially P to the plants, can increase growth and flowering rate. In wheat inoculation with P. cepacia R85, R55, P. aeuroginosa R80, R61, P. putida R104 and P. fluorescens R22 bacterial biofertilizers increased grain yield 37-78% compared to control [2]. Phosphate soluble bacteria's increased grain yield of common wheat in all researches (8). Yield and yield components of many crops increased with application of bio fertilizers (3, 4, 5). This may resulted from its ability to increase the availability of phosphorus and other nutrients especially under the specialty of the calcareous nature of the soil which cause decreasing on the nutrients availability. Non application of biofertilizers treatment had lowest grain yield, on the other hand, the lower yield in this treatment may be due to the absence of nitrogen fixation and phosphate solubilizing bacteria. It seems that nitrogen stabilization and phosphate solubilizing bacteria, by increasing yield component such as number of pods per plant, 1000 grain weight and biomass increased grain yield. For high yield, plant should have proper balance between vegetative and reproductive growth, and developmental stages of seeds completely [27, 28].

In final our results showed that combined application of Nitroxin with Soybean special Phosphate Barvar2 had a highest biofertilizers increased grain field as results of yield components and for achieved to high yield in soybean in Kangavar region we can proposed this combined fertilizer for farmers.

CONCLUSION

The present study indicates that the benefits of biofertilizers can be best exploited if they are applied with together as combination bioertilizers specially for achieved to high grain yield in soybean we must be applied combination of Nitroxin with soybean special Phosphate Barvar2 biofertilizers as well due to synergistic effect of them. Therefore, we can conclude that combined application of these fertilizers had more efficiency because of some positive interaction between their microorganisms result to synergistic effect and increase in yield components and in final grain yield. Then we can propose this biofertilizer combination to high yield in soybean for farmers of this region.

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