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Effect of Phosphate Biofertilizer and Chemical Phosphorus on Growth and Yield of *Vicia faba L.*

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Research Article

Abstract

The contribution of two phosphate biofertilizer was examined at three levels of triple supper phosphate fertilization for *Vicia faba* in a field experiment. The experiment was laid out in a factorial arrangement based on randomized complete block design with three replications. The first factor was three amounts of phosphorous chemical fertilizer (0, 100 and 150 kg ha^{-1}). The second factor was the combination of two phosphate biofertilizer (control, Barvar2, Biophosphorus and Barvar2+Biophosphorus). Analysis of variance indicated significant effects of chemical phosphorus and interaction effects of two factors on number of pods per plant, plant height, hundred seed weight, biological yield, seed yield, harvest index and protein content. The effect of phosphate biofertilizer was significant on number of pods per plant, plant height and hundred seed weight. In plots with 100 kg P ha^{-1}, the highest values of seed yield were obtained by application of Biophosphors and Barvar2+Biophosphors. Unlike these two conditions, by application of 150 kg P ha$^{-1,}$ the highest value of seed yield was obtained in un-inoculated biofertilizer treatment. The results indicated Application of chemical phosphorus fertilizer decreased to 25% by integrating biological and chemical phosphorus fertilizers without yield loss. Therefore, biofertilizer can be raised as an alternative chemical fertilizer in faba bean and application of two biological phosphorus fertilizers (combination of Barvar2 and Biophosphorus) with 100 kg ha^{-1} chemical phosphate fertilizer in addition to yield increasing could be a strategy to obtain sustainable agriculture.

Keywords: *Bacillus lentus;* Faba bean*;* Phosphorus fertilizer*; Pseudomonas putida*

1. Introduction

Faba bean (*Vicia fabae* L.) is used for human food as a green vegetable or dried, fresh or canned in the Middle East, Mediterranean region, China and Ethiopia [1]. Moreover, Faba bean also contains a chemical called Lovodopa which is used for controlling the brain neurons disease that affects body movement (Parkinson's symptoms disease) [2].

In Faba bean farming, one of the principle production restrictions is poor fertility especially nitrogen deficiency and phosphorus unavailability. Therefore, chemical fertilizers are considered a limiting factor. Some of researchers indicated beneficial of phosphorus fertilizer on increase of Faba bean yield and growth. Gizawy et al. [3] revealed that application of chemical phosphorus fertilizer had a significant effect on seed yield, yield components and content of nitrogen, phosphorus and zinc in the Faba bean seed. Rafat et al. [4] indicated that application of phosphorus at the rate of 50 kg P ha^{-1} lead to maximum values of plant height, pod length, number of pods per plant and pod yield in common bean. Adhami et al. [5] reported that dry weight of broad bean increased by application of phosphorus. They had stated increased P uptake, plant nutrition development and possibly further rooted in the soil can be reason of increasing weight of dry air organs. Turk et al. [6] indicated the significant increase of seed yield; seed weight plant^{-1}, 100 seeds weight, seeds pod^{-1}, plant height, pod length, pods plant^{-1} and branches plant^{-1} with P application. Turk et al. [7] indicated at low rates of applied P, Faba bean expressed some P deficiency symptoms, such as dwarf growth and purpling of the leaves, while such effects were absent at high P rates. Significant increases were achieved in Faba bean yield and its attributes by increasing phosphorus fertilization rate up to 45-46.5 kg P_2O_5/fed, 30-31 kg P_2O_5/fed or 22.5 kg P_2O_5 /fed [8-11].

Applications of chemical fertilizer for increase crop yield are also largely affecting environment, normal flora of soil and human health. For this reason, seed inoculation with phosphate solubilizing bacteria (PSB), nitrogen fixing microorganisms and other beneficial microorganisms as a bio-fertilizer, is an acceptable alternative to chemical fertilizer application. Biofertilizers were increasing soil fertility, while reduce the environmental impact of chemical fertilizers [12]. The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are the most powerful phosphate solubilizers [13]. Keneni et al. [14] indicated Faba bean have a potential to form association with phosphate solubilizing bacteria. Potent PSB including *Pseudomonas* sp., *Rhizobium* sp. and *Bacillus* sp. are mainly solubilized phosphate pool by production of organic acid which is easily uptake by the plant. These microorganisms can play an important role in improving yield, yield components, phosphate uptake efficiency and plant growth by releasing phosphorus from tri-calcium phosphate or rock [15].

Many investigators reported that phosphate-dissolving bacteria enhance crop growth, improve seed and straw yields and increase nutrient uptake of Faba bean [8-11]. Alipour et al. [16] indicated chemical and biological phosphorus fertilizers significantly influenced seed and biological yield, harvested index and plant height. Demissie et al. [17] indicated plant height, root length, phosphorus content, P uptake and nodule number and weight were enhanced due to inoculation with phosphate solubilizing bacteria. Mathur et al. [18] indicated Faba bean root colonization by indigenous arbuscular mycorrhizal fungi (AMF) increased vegetative growth and seed yield in addition to improving nodulation. Najjar et al. [19] evaluated the single and combined application of *Pseudomonas fluorescens* (PF, strain DR54) and arbuscular mycorrhizal fungi in terms of their effects on phosphorus (P) nutrition of plants supplied with rock phosphate (RP) in faba bean and maize and revealed beans were more efficient than maize in using P from rock phosphate. Stajković et al. [20] showed that Rhizobium co-inoculation with Pseudomonas or Bacillus strains improved shoot dry weight, nitrogen and phosphorus contents in bean plants, compared to inoculation with Rhizobium alone, whereby Pseudomonas promoted bean growth and particularly P uptake more efficiently than Bacillus.

The purpose of this research was studying the effects of various levels of phosphorus fertilizer and phosphate solubilizing bacteria on yield and yield components of Faba bean.

2. Materials and Methods

This experiment was conducted at Behshar (longitude, 53° 44´ E; latitude, 36° 42´ N; altitude, 14 m above sea level), Iran, in 20132014- growing season. The growing season rainfall was 425 mm. The experiment was laid out in a factorial arrangement based on randomized complete block design with three replications. The first factor was three amounts of phosphorous chemical fertilizer (triple supper phosphate) consisting of control (no chemical fertilizer), 100 and 150 kg ha^{-1}. The second factor was the combination of two phosphate biofertilizer (control, Barvar2, Biophosphorus and Barvar2+Biophosphorus). Barvar2 is a commercial phosphate biofertilizer containing *Pantoea agglomerans* (p5) and *Pseudomonas putida (p13). Pantoea agglomerans* (p5) associated with the production of organic acids, are causing the release of phosphate from inorganic compounds. *Pseudomonas putida (p13)* produced and secreted alkaline phosphatase enzyme and releases the phosphate from organic compounds. Biophosphorus is another commercial phosphate biofertilizer included from combination of different strains of *Bacillus* sp., *Rhizobium* sp. and *Pseudomonas* sp. In addition, use of this biofertilizer reduced local pH and provides the plants needs to zinc and iron. Use of it gradually increases the vital activity of microorganisms, modifying the physical and chemical properties of soil and root environment, increase soil fertility and improve the nutritional conditions.

Faba bean seeds (cv. Barakat) were treated with phosphate biofertilizers. The inoculation of seeds was done at the beginning of day and planting was performed after drying seeds in shade. The Faba bean was planted at 17 plants m^{-2} density (30 × 20 cm spacing distance). All the seeds were sown in experimental plots of 2.5 × 4 m in dimensions. Soil properties were presented in Table 1. Seedbed preparation was done in October. All plots were supplied with urea (100 kg ha^{-1}) as a basal application. Nitrogen fertilizer was top dressed in three portions, one third at the time of planting, one third before flowering and the remain at the time of seed filling. Other cultural practices were kept the same as normally practiced farmers in Faba bean fields. At harvest, plants were taken at random from the central ridge to determined number of pods per plant, plant height, hundred seed weight, biological yield, seed yield, harvest index and protein content

All the data were subjected to statistical analysis using SAS software [21]. Where the F-test showed significant differences among means, differences between the treatments were performed by Least Significant Differences (LSD) test.

3. Results and Discussion

As shown in Table 1, the experimental soil is clay

Table 1. Some of soil characteristics in experimental field.

Absorbable potassium (ppm)	Absorbable Zinc (ppm)	Acidity or PH	Electrical conductivity (dS m^{-1})
416	0.67	6.53	1.76
Absorbable phosphorus (ppm)	Nitrogen (%)	Carbon (%)	Tissue
2.52	0.16	1.6	Clay sand

sand with good organic matter. Available phosphorus is less than the critical level of soil (12 $mg.kg^{-1}$ of soil), which seems that the soil is appropriate to study the effect of chemical phosphorus and phosphate biofertilizer. Other factors did not limit the soil for Faba bean cultivation.

3.1 Plant height

Analysis of variance indicated the effects of phosphorus fertilizer, phosphate biofertilizer and interaction effects of two factors were significant on plant height (Table 2).

Results revealed that phosphate solubilizing bacteria increased plant height in comparison to control. LSD values for phosphate biofertilizer alone or in combination with P as described in Table 3. In 0, 100 and 150 kg ha^{-1} phosphorus fertilizer rates, the highest value of plant height were observed in Barvar2+Biophosphors (127.25 cm), Barvar2 (148.75 cm) and Biophosphors (158.7 cm), respectively. Therefore, phosphate biofertilizer enhanced positively the height of plants (Table 3). In agreement with the result of present study, the other researchers reported that phosphate-dissolving bacteria enhance plant height [3,16,17].

3.2 Number of pods per plant

Analysis of variance indicated that the number of pods per plant was significantly affected by phosphorus fertilizer, phosphate biofertilizer and interaction effects of two factors (Table 2). In 0, 100 and 150 kg P ha^{-1} rates, the maximum number of pods per plant (21.8, 28.3 and 25.83) was recorded by Barvar2, Barvar2+Biophosphors and Biophosphors, respectively, while minimum numbers was recorded in un-inoculated condition (Table 2). This indicated that in all of the phosphorus fertilizer rates, inoculation with any kinds of phosphate biofertilizer enhanced the number of pods per plant.

3.3 Hundred seed weight

Hundred seed weight was significantly affected by biological and chemical phosphorus fertilizers. The interaction effects of two factors had also significant effect on hundred seed weight (Table 2). In non P treatments, application of Barvar2+Biophosphors

Table 2. Analysis of variance for yield and yield components.

	Mean Square							
SOV	**df**	**NPP**	**PH**	**HSW**	**BY**	**SY**	**HI**	**PC**
R	3	3.40	11.1	46	1.66*	0.4*	0.015**	0.0018
P	2	199.87**	5552**	5376.08**	26.11**	12.97**	0.016**	0.0139**
BP	3	31.84*	365.6**	297.55**	0.58	0.15	0.004	0.0013
P*BP	6	25.15*	435.2**	1086.97**	3.61**	0.44**	0.008**	0.0034**
Error	33	9.79	28.6	59.33	0.43	0.07	0.002	0.0008
CV		13.42	3.99	4.48	7.71	6.8	10.09	12.80

*, ** significant at 5% and 1% probability levels, respectively

P: Chemical Phosphorus; BP: Phosphate Biofertilizer; NPP: Number of Pods Per Plant; PH: Plant Height; HSW: Hundred Seed Weight; BY: Biological Yield; SY: Seed Yield, HI: Harvest Index; PC: Protein Content; CV: Coefficient of Variation

Table 3. Simple effects of phosphate biofertilizer on studied traits in any level of chemical phosphorus.

P (Kg ha^{-1})	Phosphate biofertilizer	NPP	pH (cm)	HSW (g)	BY (ton ha^{-1})	SY (ton ha^{-1})	HI (ratio)	PC (%)
0	control	13.7b	106.75c	125d	6.07c	2.5b	0.37c	0.20
	Barvar2	21.8a	113.87b	157c	7.5ab	3.32a	0.45ab	0.20
	Biophosphors	18.9ab	109.50 bc	142b	6.25bc	3.15a	0.5a	0.20
	Barvar2+ Biophosphors	22.3a	127.25a	179.5a	8.47a	3.1a	0.42bc	0.20
	LSD (5%)	5.58	5.54	11.85	1.42	0.52	0.07	-
100	control	22.7b	127.13b	188a	9.1bc	4.2b	0.47ab	0.20a
	Barvar2	23.87b	148.75a	187.5a	9.45b	4.3b	0.45b	0.22a
	Biophosphors	23.53b	146.75a	177.5a	10.22a	4.72a	0.47ab	0.2a
	Barvar2+ Biophosphors	28.3a	123b	180.5a	8.92c	4.72a	0.52a	0.22a
	LSD (5%)	4.06	7.59	12.3	0.52	0.37	0.06	0.05
150	control	24.9a	144c	182.5a	9.92a	4.95a	0.47a	0.27a
	Barvar2	25.3a	146.6bc	177.5a	8.6b	4.75ab	0.55a	0.20b
	Biophosphors	25.82a	158.7a	182.5a	8.77b	4.42b	0.50a	0.3a
	Barvar2+ Biophosphors	25.32a	156.1a	173.5a	9.17ab	4.5b	0.47a	0.25ab
	LSD (5%)	3.88	11.7	11.01	0.95	0.41	0.08	0.06

P: Chemical Phosphorus; BP: Phosphate Biofertilizer; NPP: Number of Pods Per Plant; PH: Plant Height; HSW: Hundred Seed Weight; BY: Biological Yield; SY: Seed Yield; HI: Harvest Index; PC: Protein Content increased hundred seed weight to 179.5 g which was 30% higher as compared to control. While in P applied condition, application of phosphate biofertilizer did not significant effect on hundred seed weight and there were not significant differences between inoculated and un-inoculated treatments (Table 3). The maximum values of hundred seed weight were obtained by application of 100 kg P ha^{-1} (188 g). Turk et al. [6] was also indicated that hundred seed weight influenced by phosphorus fertilizer.

3.4 Seed yield

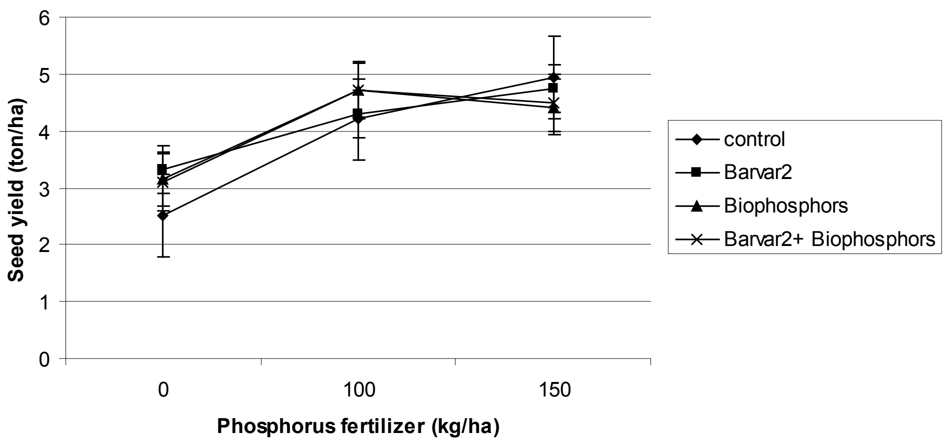
Analysis of variance indicated that seed yield was affected by phosphorus fertilizer, phosphate biofertilizer and interaction effects of two factors (Table 2). According to these results, highest (4.95 ton ha^{-1}) and lowest (2.5 ton ha^{-1}) values of seed yield were obtained by application of 150 kg P ha^{-1} and control treatments, respectively. In non P application conditions, the highest value of seed yield (3.32 ton ha^{-1}) was obtained by Barvar2 inoculation. In plots with 100 kg P ha^{-1}, the highest values of seed yield were obtained by application of Biophosphors and Barvar2+Biophosphors. Unlike these two conditions, by application of 150 kg P ha^{-1}, the highest value of seed yield were obtained in un-inoculated phosphate biofertilizers (Table 3). These findings indicate up to 100 kg P ha^{-1}, Faba bean seed yield increased by application of biological phosphate fertilizer. These findings are also supported by other researchers [8-11,16,17,19]. It could be due to increasing other nutrient absorption and in increasing available phosphorus of insoluble phosphorus sources. Biological phosphate fertilizer can also be used as a solution for increasing phosphate and micronutrients sorption in the alkaline soil [22]. Increase of seed yield under the influence of biological phosphate fertilizer +100 kg P ha^{-1} can be attributed to the ability of phosphate solution bacteria in bio-fertilizer in increasing available phosphorus of insoluble phosphorus sources. In another study, Rokhzadi et al. [23] reported that seed yield of chickpea increased by application of biological fertilizers. *Pseudomonas* bacteria in the bio-phosphate produced auxin and gibberellin hormones and vitamins, therefore dry matter increasing could be attributed to the ability of bacteria [22]. The mean comparisons of seed yield were also indicated in Figure 1 and shows that P application is important nutritional element to get better Faba bean yield. The similar results were observed by El-Gizawy et al. [3], Adhami et al. [5], Turk et al. [6], Yilmaz et al. [24]. Khan et al. [25] reported that P application and Rhizobium inoculation significantly increased pod formation, seed yield and dry matter production in mung bean as compared with un-inoculated treatments. Alipour et al. [16] reported the highest seed yield (3.41 tons ha^{-1}) observed in treatments using inorganic fertilizer (35 Kg ha^{-1} super phosphate triple+100 Urea Kg ha^{-1})+nitrogen and phosphorus bio-fertilizers.

3.5 Seed protein

Percent of seed protein was significantly affected by chemical phosphorus fertilizers and interaction effects of two factors (Table 2). Mean comparisons indicated that maximum seed protein (0.30%) was obtained by application of Biophosphors +150 kg P ha^{-1} (Table 3). Phosphate solubilizing microorganisms increased available phosphorus and nitrogen in the soil which increases their concentration by plant, also increased the seed protein [26]. These results are consistent with the view of Galavi et al. [27] that indicated phosphate solubilizing microorganisms increased the grain protein on the maize. Stajković et al. [20] showed also Rhizobium co-inoculation with Pseudomonas or Bacillus strains improved shoot dry weight, nitrogen and phosphorus contents in bean plants.

3.6 Biological yield

Analysis of variance indicated biological yield was significantly affected by chemical phosphorus fertilizers and interaction effects of two factors (Table 2). Maximum biological yield (10.22 ton ha^{-1}) was obtained by application of Biophosphors +100 kg P ha^{-1} which was 40% higher as compared to control treatment (no chemical and biological fertilizers) (Table 3). Alipour et al. [16] and El-



Vertical bars represent ± 1 SE of means.

Figure 1. Chemical phosphorus × phosphate biofertilizer interaction effect on seed yield.

Habbasha et al. [8] findings also supported the results that the biological yield increase was higher in P-treated plants inoculated with biofertilizer than the plants inoculated with bacteria alone. Phosphate-solubilizing microorganisms had important role in phosphorus solubility and uptake. The bacteria released phosphorus from organic and inorganic soils during the mineralization process also increased the absorption of phosphorus from phosphate rock and save a reservoir of phosphorus in the presence of carbon unstable by mineralization process [28].

3.7 Harvest index

Phosphorous fertilizers and interaction effects of two factors had a significant effect on harvest index (Table 2). Mean comparisons indicated that maximum harvest index (55 %) was obtained by application of Barvar2 +150 kg P ha^{-1}; also minimum harvest index (37 %) was obtained by non chemical and biological phosphorus treatment (control). Similar results were shown by Alipour et al. [16] that indicated harvested index significantly affected by using chemical and biological phosphorus fertilizers.

4. Conclusion

The results indicated application of phosphorus fertilizers combined with 100 kg P ha^{-1} chemical phosphorus increased dry matter accumulation. Biological phosphorus fertilizers efficiency increases if combined with 75% chemical phosphorus fertilizers. Integrating biological and chemical phosphorus fertilizers increased seed and biological yield as compared to control. Biophosphors with 150 kg ha^{-1} chemical phosphorus fertilizer increased seed protein, because nutrients absorption capability and plant absorption balance increased by phosphate-solubilizing microorganisms. Application of biological fertilizer (Barvar2+Biophosphors) was significantly increased 100-seed weight, in the absence of chemical phosphorus, but when chemical phosphorus were applied, there were not significantly 100-seed weight influenced by phosphate biofertilizer. The beast treatment combination was 100 kg P ha^{-1} chemical phosphorus and combination of two biological phosphorus fertilizers (Barvar2+Biophosphorus). Application of chemical phosphorus fertilizer decreased to 25% by integrating biological and chemical phosphorus fertilizers without yield loss. Also environmental contamination was reduced by decreasing consumption of chemical fertilizers. Overall, application of two biological phosphorus fertilizers (combination of Barvar2 and Biophosphorus) with 100 kg ha^{-1} chemical phosphate fertilizer in addition to yield increasing could be a strategy to obtain sustainable agriculture.

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