

Green bean (*Phaseolus vulgaris* L.) growth and yield as affected by chemical phosphorus fertilizer and phosphate bio-fertilizer

Efecto del uso de fertilizantes fosfatados de origen químico y bio-fertilizantes en el crecimiento y producción de judía (Phaseolus vulgaris L.)

Faegheh Ranjbar-Moghaddam¹, Hashem Aminpanah^{*1}

ABSTRACT

A field experiment was conducted at Khomam, Guilan province, northern Iran, to evaluate the effects of phosphorus rate and phosphate bio-fertilizer on growth and yield of green bean (*Phaseolus vulgaris* L.). The experimental design was a factorial arrangement of treatments based on a randomized complete block with four replicates. Factors were phosphorus fertilizer rates (0, 25, 50, 75, and 100 kg ha⁻¹ as triple superphosphate) and phosphate bio-fertilizer application (seed inoculated with phosphate bio-fertilizer containing *Pseudomonas fluorescens* strain P5 and strain p13, and non-inoculated (control)). Analysis of variance showed that phosphorus rate and phosphate bio-fertilizer had significant effect on plant height, pod number per plant, pod yield and pod N and P concentration. The relationships between phosphorus application rate and pod number per plant and pod yield were well expressed by linear equations. Regardless of phosphate bio-fertilizer application, pod number per plant was significantly increased from 55 to 85 pods per plant as P application rate increased from 0 to 100 kg ha⁻¹. Similarly, pod yield increased linearly with increasing P application rate from 0 to 100 kg ha⁻¹. Regardless of phosphorus rate, pod number per plant and pod yield were significantly higher in plots that received phosphate bio-fertilizer compared to control plots. Pod number per plant and pod yield increased by 16% and 15%, respectively, when phosphate bio-fertilizer was applied. Based on the results of this experiment, P application at the rate of 100 kg ha⁻¹ along with phosphate bio-fertilizer are recommended for obtaining the greatest pod yield in green bean.

Keywords: phosphorus, chemical fertilizer, bio-fertilizer, green bean, *Phaseolus vulgaris* L.

RESUMEN

EL objetivo de este trabajo fue evaluar el efecto de la aplicación de fosfato con una fuente química y bio-fertilizante en el crecimiento y rendimiento de judías (*Phaseolus vulgaris* L.). El experimento se realizó en Khomam, provincia Guilan, al norte de Irán. El diseño experimental fue de bloques completos al azar con arreglo factorial 5X2 con cuatro repeticiones. Los tratamientos fueron aplicación de fósforo en dosis de 0, 25, 50, 75, y 100 kg ha⁻¹ como superfosfato triple y aplicación de fosfato bio-fertilizante con semillas inoculadas con *Pseudomonas fluorescens* y semillas no inoculadas (control). Los resultados indican que la aplicación de SPT y bio-fertilizantes afecta significativamente la altura de la planta, el número de vainas por planta, rendimiento y la concentración de N y P en vainas. Las relaciones entre dosis de fertilizantes fosfatado y bio-fertilizante se expresaron con ecuaciones lineales. Independientemente de la aplicación de bio-fertilizantes, el número de vainas aumentó significativamente de 55 a 85 vainas por planta cuando la aplicación de P aumento de 0 a 100 kg ha⁻¹. El rendimiento de vainas aumentó a mayor dosis de P de 0 a 100 kg ha⁻¹. El número de vainas y rendimiento fue significativamente mayor en las parcelas inoculadas con bio-fertilizantes en comparación con el control, para todas las dosis de fosfato. El número de vainas por planta y rendimiento se incrementó en un 16% y 15%, respectivamente, cuando se aplicó bio-fertilizante. Los resultados sugieren que la aplicación de P en dosis de 100 kg ha⁻¹ y la aplicación de bio-fertilizante es recomendable para obtener mayor rendimiento de vaina en judías.

Palabras clave: fósforo, fertilizante químico, bio-fertilizantes, judía, *Phaseolus vulgaris* L.

Introduction

Green bean (*Phaseolus vulgaris* L.), also known as snake bean, is a warm-season crop which is usually

cultivated in early May and harvested in early August in northern Iran. Worldwide, green beans are produced on 1.5 million ha, with an estimated total production of 20.7 million tons in 2012 (FAO, 2012).

¹ Department of Agronomy and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran.

* Corresponding author: aminpanah@iaurasht.ac.ir

Phosphorus (P) is the second major essential macronutrient after nitrogen, limiting plant growth and development (Feng *et al.*, 2004). Phosphorus plays a vital role in protein synthesis, photosynthesis, respiration, energy reactions, genetic transfer, cell division, development of new tissue and nutrient transport in plants (Raghothama and Karthikeyan, 2005). Phosphorus is also a component of complex nucleic acid structure, phytin, a major storage form of P in seeds, phospholipids and ATP. Moreover, phosphorus increases seed and fruit production, promotes root growth, stimulates tillering and often hastens maturity. Li *et al.* (2011) declared that leguminous crops, in general, respond better to P fertilizer than cereals and stated that the responses of leguminous crops to P fertilizer are mainly determined by the soil P available. Results show that the application of P fertilizer was required when the available P in the soil was less than 10 ppm (Lin *et al.*, 1964). In contrast, the application of P fertilizer had no consistent effect when the available P in the soil was more than 15 ppm (Lin *et al.*, 1964). In a field experiment, Li and Li (1992) reported that wheat yield increased linearly with increasing P rate from 0 to 97.5 kg ha⁻¹. In contrast, the highest pea yield was at the P rate of 78 kg P ha⁻¹, and thereafter decreased. They concluded that legume crops show higher P demand compared to non-legume crops.

Plants absorb phosphorus mostly in soluble (H₂PO₄⁻ and HPO₄²⁻) forms (Bhattacharyya and Jha, 2012). Although P is abundantly available in soils (Khan *et al.*, 2006), its availability for plants is generally low, because at least 70-90% of the P that enters the soil is fixed by Fe, Al, and Ca in soils (McBeath *et al.*, 2006). To overcome P deficiency in soils, phosphorus fertilizers must be frequently added to agricultural fields, but regular application of phosphate fertilizers increases the cost of crop production and exacerbates eutrophication problems in surface waters. Therefore, a number of intellectuals started working on an eco-friendly alternative and found that bio-fertilizers can help to increase yield without causing the damage associated with chemical fertilizers. *Pseudomonas fluorescens* is considered as one of the most significant phosphate-solubilizing bacteria, which not only enhances P availability for plants (Shaharoon *et al.*, 2008), but also produces siderophore (Shaharoon *et al.*, 2008; Braud *et al.*, 2009), antifungal metabolites (Saravanakumar *et al.*, 2007; Dey *et al.*, 2004),

1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme (Shaharoon *et al.*, 2008) and phytohormones such as indole-3-acetic acid (Dey *et al.*, 2004). Grimes and Mount (1984) found that legume growth, nodulation, and nitrogen fixation enhanced when some PGPR strains such as *Pseudomonas* co-inoculated with rhizobia. Van Elsas (1986) reported that seed inoculated plants with *Pseudomonas* produced greater biomass than uninoculated plants. Phosphate bio-fertilizer application has been reported to increase P availability for plants via the processes of acidification, chelation, exchange reactions and production of gluconic acid (Chung *et al.*, 2005; Gulati *et al.*, 2010), and may reduce chemical phosphorus fertilizer rate (Khan *et al.*, 2006). Adesemoye *et al.* (2009) found that supplementing 75% of the recommended fertilizer rate with inoculants produced plant growth, yield, and nutrient (nitrogen and phosphorus) uptake that were statistically equivalent to the full fertilizer rate without inoculants. Therefore, this experiment was conducted to evaluate the effects of phosphorus fertilizer rate and phosphate bio-fertilizer on green bean growth and yield.

Materials and Methods

Experimental site and design, crop management and sampling

A field experiment was carried out at Khomam, Guilan province, northern Iran, in 2014. Some soil properties of the experimental field are presented in Table 1. Monthly air temperature, and precipitation during the green bean growing period were measured in the weather station of Bandar-e-Anzali, located 25 Km from the experimental site (Table 2). Treatments consisted of a factorial combination of five phosphorus fertilizer rates (0, 25, 50, 75, and 100 kg ha⁻¹ as triple superphosphate) and two phosphate bio-fertilizer applications, seed inoculated and non-inoculated (control). Phosphate bio-fertilizer consisted of *Pseudomonas fluorescens* strain P5 and strain p13. Treatments were arranged in a randomized complete block design with four replicates. Just before final land preparation, 15 kg N ha⁻¹ (as starter in the form of urea), and 50 kg K₂O ha⁻¹ (as potassium sulfate) were applied to the plots. Green bean seeds were inoculated with phosphate bio-fertilizer before planting and dried in the shade for five hours. Plots (2 m wide

Table 1. Some soil properties (0-30 cm) of experimental field prior to sowing.

OC (%)	PH	Sand (%)	Silt (%)	Clay (%)	Texture	EC (ds m ⁻¹)	Total N (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)
2.3	7.8	40.8	42.0	17.2	Loam	0.61	0.11	8.4	188

Table 2. Monthly precipitation, maximum, minimum and average temperature during the green bean growing period in northern Iran.

Month	Year	Precipitation (mm)	Temperature (°C)		
			Maximum	Minimum	Average
May	2014	18.5	24.9	19.4	22.5
June	2014	29.2	28.0	25.8	22.0
July	2014	16	29.7	24.2	26.9
August	2014	74.5	31.5	24.8	28.2

× 4 m long) were hand-seeded on 5 May using row spacing of 50 cm at a plant density of 10 plants m⁻². To provide the target plant population density, two seeds per hill were sown and plants were thinned to one per hill when the second leaf emerged. The plots were irrigated as necessary throughout the season to avoid water stress. Weeds were hand-controlled four times during the experiment i.e. on 22 May, 3, 18, and 30 June 2014. Since the crop was not affected by pests or diseases, no pesticides were used during the experiment. Plant height was measured from the soil surface to the top of the main stem at 55 and 85 days after sowing (first and final pod harvesting stages, respectively). Pod number per plant was determined from ten randomly selected plants in each plot. Pods were harvested from 2 m of the two center rows of each plot on 29 June, 9, 19 and 30 July 2014. Pods were dried at 72 °C for 96 h, ground to pass through a 1-mm sieve and N, P were measured. N concentration was determined using the micro-Kjeldahl method following salicylic-H₂SO₄ digestion (Yamakawa, 1993) and P concentration was determined using the spectrophotometric method of Lowry and Lopez (1946).

Statistical analyses

Analysis of variance was conducted using PROC GLM of SAS (SAS Inst., 2004). The analysis was calculated as a factorial treatment arranged in a randomized complete block design. For phosphate bio-fertilizer factor, where the F-ratios were found to be significant, separation of means was performed using Fisher's protected LSD at

the 5% probability level. For P rate factor, where the F-ratios were found to be significant, linear or quadratic regressions with standard error of the mean were used to describe the relationship between P application rate and dependent variables such as plant height, pod number per plant, pod yield and pod N and P concentration.

Results and Discussion

Plant height

Analysis of variance showed that there was a significant P rate effect on plant height at 55 and 85 days after sowing (first and final harvesting stages, respectively), while the main effect of phosphate bio-fertilizer and the interaction between P rate and phosphate bio-fertilizer were not significant (Table 3). The relationships between P application rate and plant height at 55 and 85 days after sowing were well fitted by quadratic equations. Plant height at 55 days after sowing was significantly increased from 39cm to 48cm as P rate increased from 0 to 50 kg ha⁻¹, and thereafter relatively remained constant (Figure 1). Similarly, plant height at 85 days after sowing was significantly increased from 46cm to 55cm as P rate increased from 0 to 50 kg ha⁻¹, but relatively remained constant at higher P rates (Figure 1). Similar results were reported in soybean (Shahid, *et al.*, 2009). In contrast, Turuko and Mohammed (2014) reported that P rate had no significant effect on plant height in common bean (*Phaseolus vulgaris* L.). The promotion effect of high P level on plant height may be due to better

Table 3. Mean squares of ANOVA for plant height at 50% flowering stage (Hf), plant height at final harvesting stage (Hfh), pod number per plant (PN), pod yield (PY), pod N, P and K concentrations (PNC, PPC and PKC, respectively) as affected by phosphorus rate and phosphate bio-fertilizer.

S.O.V	df	H _f	H _{fh}	PN	PY	PNC	PPC	PKC
R	3	13 ^{ns}	13 ^{ns}	42 ^{ns}	508469 ^{ns}	0.06 ^{ns}	0.000001 ^{ns}	0.005 ^{ns}
Phosphorus rate (P)	4	199 ^{**}	136 ^{**}	817 ^{**}	137019831 ^{**}	0.62 ^{**}	0.0054 ^{**}	0.118 [*]
phosphate bio-fertilizer (Pb)	1	46 ^{ns}	41 ^{ns}	803 ^{**}	78806525 ^{**}	1.52 ^{**}	0.0001 ^{ns}	0.031 ^{ns}
P × Pb	4	25 ^{ns}	7 ^{ns}	38 ^{ns}	7143379 ^{ns}	0.56 ^{**}	0.0055 ^{**}	0.127 [*]
Error	27	27	10	52	3178794	0.04	0.0004	0.038
CV (%)	-	10.9	6.5	10.8	9.2	10.0	9.2	8.1

*, ** represent significance at 0.05 and 0.01 probability levels, respectively. ns represents non-significant difference.

Table 4. plant height at 50% flowering stage (Hf), plant height at final harvesting stage (Hfh), pod number per plant (PN), pod yield (PY), pod N, P and K concentrations (PNC, PPC and PKC, respectively) response to phosphate bio-fertilizer.

Traits	H _f	H _{fh}	PN	PY	PNC	PPC	PKC
Biofertilizer application	48.2	54.2	72.5	20716.8	2.04	0.22	2.42
Without biofertilizer application	46.1	52.2	62.5	17909.5	1.65	0.21	2.37
LSD (0.05)	3.3	2.1	4.7	1156.8	0.12	0.13	0.12

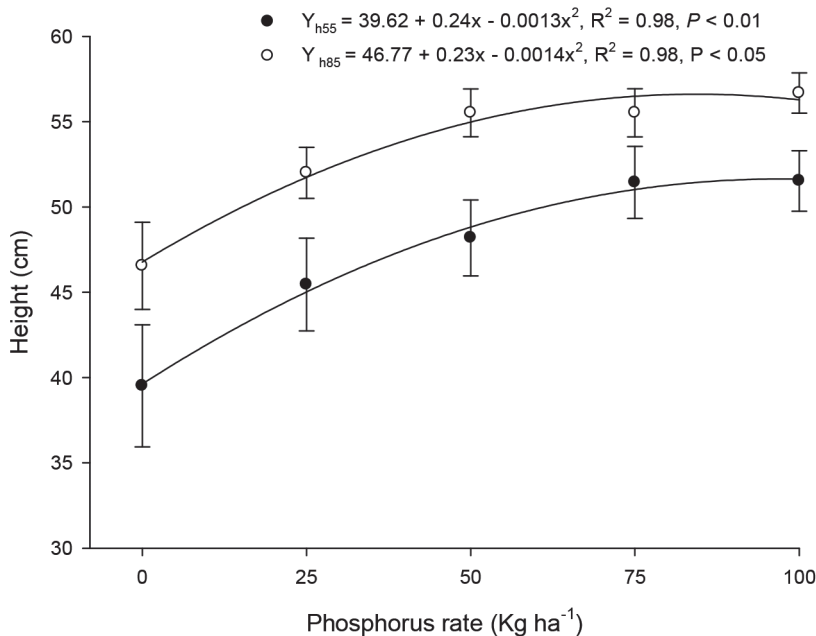


Figure 1. Effect of phosphorus fertilizer rate on plant height at 55 and 85 days after sowing (h55 and h85, respectively) averaged across phosphate bio-fertilizer application. Vertical bars represent ± 1 SE of means.

development of the root system and nutrient absorption (Hussain *et al.*, 2006). Regardless of phosphorus rate, plant height at 55 and 85 days after sowing were not significantly increased due to phosphate bio-fertilizer

application (Table 4). Contrary to this result, Dey *et al.* (2004) reported that plants from seed inoculated with *Pseudomonas fluorescens* were significantly taller than control plants.

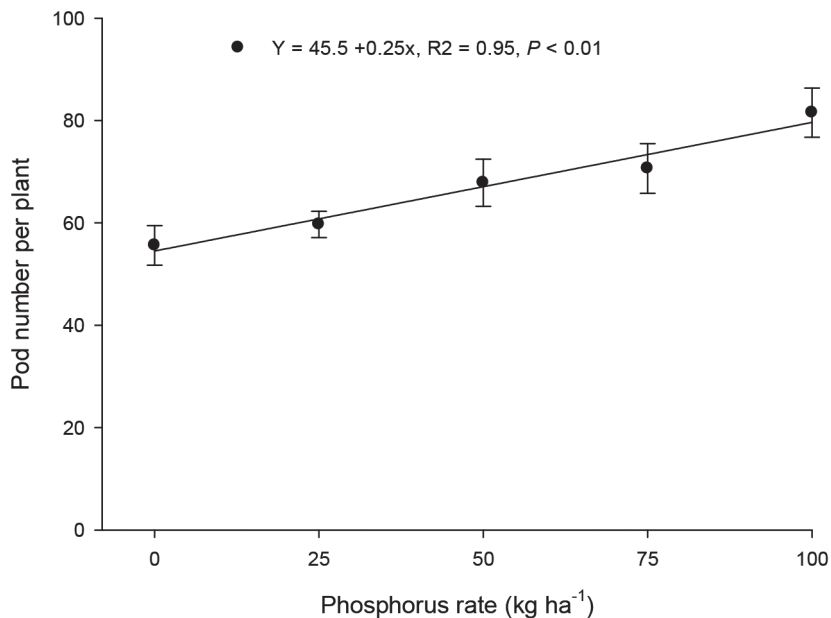


Figure 2. Effect of phosphorus fertilizer rate on pod number per plant averaged across phosphate bio-fertilizer application. Vertical bars represent ± 1 SE of means.

Pod number per plant

Pod number per plant was significantly influenced by the main effects of P rate and phosphate bio-fertilizer, while the interaction between them was not significant (Table). A linear equation provided a good description of the relationship between pod number per plant and phosphorus rate. Regardless of phosphate biofertilizer application, pod number per plant was significantly increased from 55 to 85 pods per plant as P application rate increased from 0 to 100 kg ha⁻¹ (Figure 2). These results are similar to those of Turuko and Mohammed (2014), who reported that the highest pod number per plant was recorded with P rates of 20 kg ha⁻¹. This may be due to the fact that P increases flower formation and improves fruit setting. Pod number per plant was significantly higher (16%) in seed-inoculated plants compared to control plants (Table 4). These results confirm the earlier findings of Dey *et al.* (2004) in peanut (*Arachis hypogaea* L.).

Pod yield

The main effects of P rate and phosphate bio-fertilizer were significant for pod yield, while P

rate \times phosphate bio-fertilizer interaction was not significant (Table 3). The relationship between P application rate and pod yield was well expressed by a linear equation. Pod yield increased significantly with increasing P application rate (Figure 3). Phosphorus (P) is an essential macronutrient which affects plant growth and yield through the following: 1) P promotes root growth and stimulates lateral branching which increases plant ability to absorb nutrients from the soil, 2) Phosphorus plays a vital role in flower formation and fruit setting, 3) Phosphorus is a crucial element in legume crop production which plays an important role in many processes such as sugar and starch utilization, photosynthesis, cell division and organization and nodule formation, 4) Phosphorus increases leaf area index through increases in leaf cell division, elongation and leaf number (Assuero *et al.*, 2004; Kavanova' *et al.*, 2006), thus it increases light interception and photosynthesis assimilates and therefore increases plant biomass accumulation, 5) Also, phosphorus improves nitrogen metabolism in plants (Li and Zhao, 1990). Regardless of the phosphorus rate, pod yield was significantly greater (15%) for plots that received phosphate bio-fertilizer compared to control plots (Table 4). The promotion

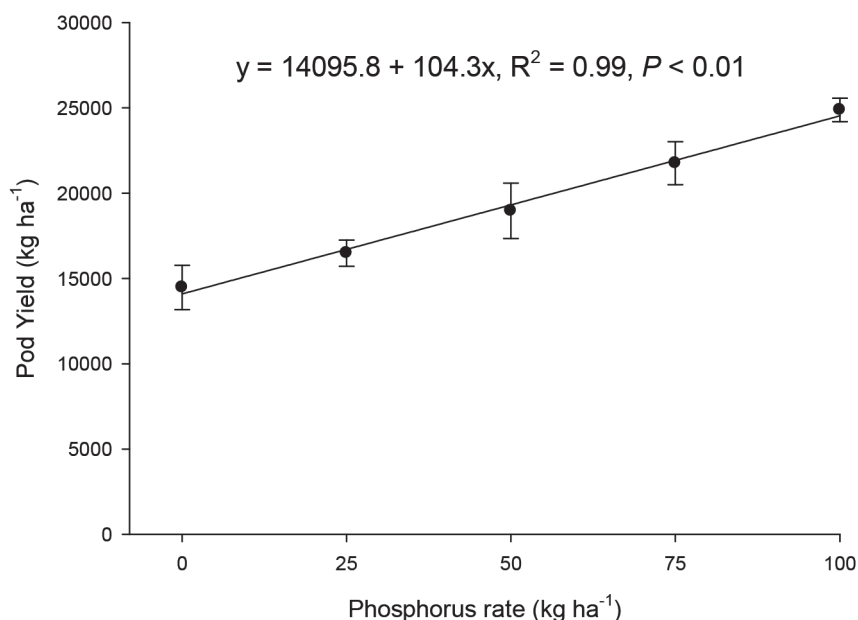


Figure 3. Effect of phosphorus fertilizer rate on pod yield averaged across phosphate bio-fertilizer application. Vertical bars represent ± 1 SE of means.

effect of *Pseudomonas* strains on plant growth and production is attributed to increased P availability for plants (Chung *et al.*, 2005; Gulati *et al.*, 2010), siderophore production (Dey *et al.*, 2004), ACC-deaminase production (Dey *et al.*, 2004; Shaharoon *et al.*, 2008) and phytohormone production such as indole-3- acetic acid (Leinhos and Nacek, 1994; Dey *et al.*, 2004).

Pod N concentration

P rate, phosphate bio-fertilizer, and P rate \times phosphate bio-fertilizer interaction had significant effect on pod N concentration (Table 3 and Table 4). For both with and without phosphate bio-fertilizer applications, a positive linear equation expressed the relationship between P application rate and pod N concentration. However, pod N concentration increased more rapidly when phosphate bio-fertilizer was applied compared to when phosphate bio-fertilizer was not applied. Moreover, for 25, 50, 75, and 100 kg P ha⁻¹, pod N concentration was significantly higher when phosphate bio-fertilizer was applied compared to when phosphate bio-fertilizer was not applied (Figure 4). These results support the findings of Dwivedi and Bapat (1998), who reported that nitrogen content in soybean

increased significantly by P application up to 50 kg ha⁻¹. Nitrogen and phosphorus are closely related in crop nutrition. The supply of nitrogen is necessary to allow crops to use phosphorus (Li and Zhao, 1990), while the metabolism of nitrogen is inhibited with an inadequate supply of phosphorus.

Pod P concentration

The bio-fertilizer main effect was not significant ($P > 0.05$), while the P rate main effect was significant ($P < 0.01$) for pod P concentration (Table 3 and Table 4). Moreover, the phosphate bio-fertilizer \times P rate interaction was significant ($P < 0.01$). For both with and without phosphate bio-fertilizer applications, the relationship between P application rate and pod P concentration was well fitted by linear equations. Pod P concentration was significantly increased with increasing P application rate (Figure 5). Similar results were reported by Singh and Singh (2004) in black gram and Chandra Deo and Khaldelwal (2009) in chickpea, who reported that P content in grain and straw was significantly increased with increasing in P application rate. When P fertilizer was applied at the rate of 0 and 25 kg P ha⁻¹, Pod P concentration in plots that received phosphate bio-fertilizer was significantly greater than pod P

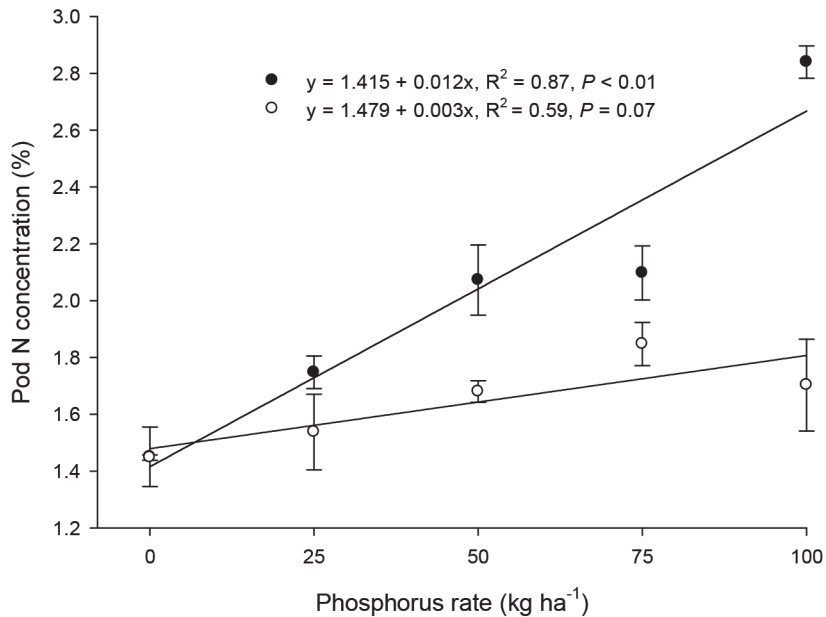


Figure 4- phosphorus fertilizer rate × phosphate bio-fertilizer interaction effect on pod N concentration. Vertical bars represent ± 1 SE of means.

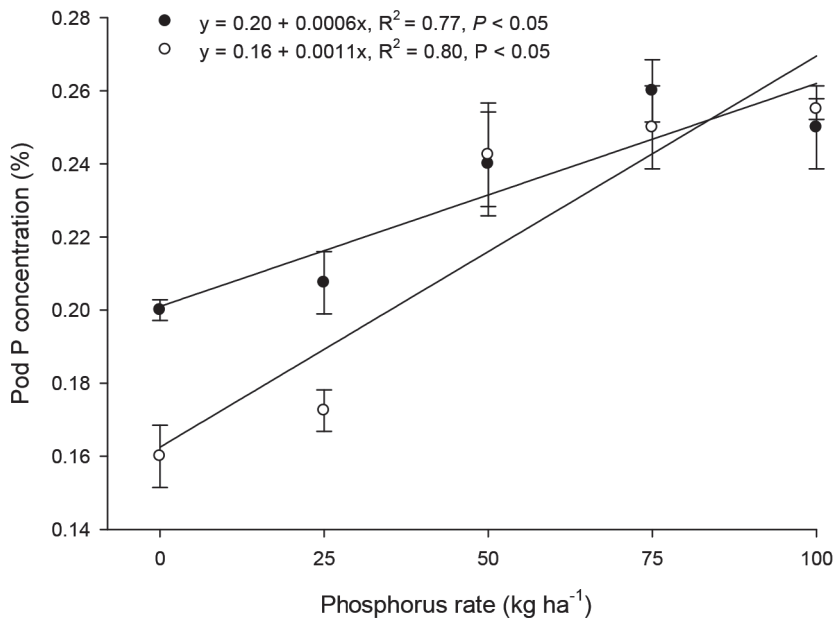


Figure 5. phosphorus fertilizer rate × phosphate bio-fertilizer interaction effect on pod P concentration. Vertical bars represent ± 1 SE of means.

concentration in plots that received no phosphate bio-fertilizer (Figure 5). Moreover, Dey *et al.* (2004) reported that seed inoculation with some *P.*

fluorescens strains significantly enhanced the total phosphorus content in shoots of peanut (*Arachis hypogaea* L.).

Conclusions

This experiment indicated that pod yield was significantly increased from 14472.4 kg ha⁻¹ to 24881.3 kg ha⁻¹ as P application rate increased from 0 to 100 kg ha⁻¹. Thus plots that received 100 kg P ha⁻¹ produced 71% greater pod yield than

those that received no P fertilizer. At the same time, pod yield was increased by 15% when phosphate bio-fertilizer was applied. Based on the results of this experiment, P application at the rate of 100 kg ha⁻¹ along with phosphate bio-fertilizer are recommended for obtaining the greatest pod yield in green bean.

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