Effects of Phosphorus Rate and Iron Foliar Application on Green Bean (*Phaseolus vulgaris* L.) Growth and Yield

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Summary

The aim of this experiment was to evaluate the effects of phosphorus rate and iron foliar application on growth and yield of green bean (*Phaseolus vulgaris* L.). A field experiment was conducted at Roudsar, northern Iran, from mid-March to early September of 2013. Factors were the iron foliar application rate (0, 1, 2, 3, 4 g L^{-1} of Sequestrene 138Fe, Iron Chelate 6%) and phosphorus fertilizer rates (0, 50, and 100 kg P_2O_5 ha^{-1} as triple superphosphate). Treatments were assigned in a factorial arrangement in a randomized complete block design with three replicates. ANOVA indicated that pod yield and pod number per plant were significantly influenced by both phosphorus fertilizer rate and iron foliar application. Results showed that total pod yield was increased by 32% when P application rate increased from 0 to 100 kg ha^{-1}. At the same time, pod yield was increased by 46% as Fe foliar application rate increased from 0 to 2 g L^{-1} and thereafter relatively remained constant. Based on the results of this study, P application at the rate of 100 kg ha^{-1} and Fe foliar application at the rate of 2 g L^{-1} are recommended for obtaining the highest pod yield in green bean.

Key words

chemical fertilizer, green bean (*Phaseolus vulgaris* L.), micronutrient, spray

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Introduction

In the developing countries, leguminous crops serve as the major source of protein in the diet. Green bean (*Phaseolus vulgaris* L.), also known as the snake bean, is a warm-season annual legume crop grown for their edible fresh pods. In northern Iran, it is usually grown during the warmer months from spring to early autumn. Worldwide, green beans is produced on 1.5 million ha, with an estimated of total production of 20.7 million tons in 2012 (FAO, 2012).

Phosphorus (P) is one of the 16 essential elements for plant growth and reproduction (Feng et al., 2004). Plants absorb phosphorus mostly in soluble ($H_2PO_4^-$ and HPO_4^{2-}) forms (Bhattacharyya and Jha, 2012). It has been demonstrated that high rates of P fertilizer can inhibit Fe uptake in many crops. At alkaline soils, phosphate ions tend to react quickly with magnesium (Mg) and calcium (Ca) to form insoluble magnesium and calcium phosphate. To overcome the P deficiency in soils, P fertilizer must be applied in or close to the seed-row at planting time to facilitate early season uptake of phosphate ions by crop roots. Li et al. (2011) reported that good responses of leguminous crops to P fertilizer are mainly determined by the soil P available. Results show that the application of P fertilizer had positive effect on leguminous seed yield when the available P in the soil was less than 10 ppm (Lin et al., 1964). In a field experiment, Li and Li (1992) reported that the highest pea yield was obtained at the P rate of 78 kg P ha^{-1}, and thereafter decreased.

Iron (Fe) is one of the essential micronutrients for higher plant growth and reproduction (Welch, 1995). Iron plays an important role in nitrate and sulfate reduction and energy production within the plant. Although iron is not a constituent of chlorophyll, it is essential for its formation (Pushnik et al., 1984). In leguminous crops, iron plays an important role in N_2 fixation. Iron deficiency is a common yield-limiting factor for crops grown on high pH ofsoil (Franzen and Richardson, 2000). The pH of soil directly affects the uptake of iron by plants. If the pH exceeds 6.5, iron is converted to an insoluble form that cannot be absorbed by plants. It is possible to lower the pH of a soil using finely ground elemental sulphur. However, this is rarely done on a field-scale basis because this is not economically feasible. At the same time, soil application of iron fertilizers to correct iron deficiency has not been economically possible on a field scale. Foliar application of Fe solutions is one of the most widely used methods for correcting Fe deficiency in many crops. This method of application usually circumvents the problems associated with Fe application to the soil. Goos and Johnson (2000) reported that foliar sprays of FeEDTA significantly reduced iron-deficiency chlorosis, while increased seed yield in soybean. In French bean, Borowski and Michałek (2011) found that Fe foliar application significantly increased chlorophyll a+b and carotenoid content in the leaves as well as their stomatal conductance, photosynthesis and transpiration rates. Heithold et al. (2003) indicated that soybean seed yield was not significantly influenced by soil application of Fe as $FeSO_4$, FeDTPA or FeEDDHA.

Soil pH in some regions of northern Iran is high, and in these regions phosphorus and iron deficiency were observed. This experiment was conducted to evaluate the effect of phosphorus rate and iron foliar application rate on green bean growth and pod yield.

Materials and methods

Experimental site and design, crop management, and sampling

A field experiment was carried out at Roudsar, northern Iran, in 2013. Some soil properties of the experimental field are presented in Table 1. Weekly air temperature, and precipitation during green bean growing period, which were measured in the weather station of Lahijan, located at the 30 Km from the experimental site, are presented in Figures 1 and 2. Factors were the iron foliar application rates (0, 1, 2, 3, 4 g L^{-1} of Sequestrene 138-Fe, iron chelate 6%) and phosphorus fertilizer rates (0, 50, and 100 kg P_2O_5 ha^{-1} as triple superphosphate). Fe foliar application was applied twice, at 27 and 41 days after sowing, using a knapsack hand sprayer. Phosphorus fertilizer was placed in bands on either side of the row before planting. Treatments were assigned in a factorial arrangement in a randomized complete block design with three replicates. Moreover, 25 kg N ha^{-1} (as starter in the form of urea) and 100 kg K_2O ha^{-1} (as potassium sulphate) were applied to the plots before seed planting. Green bean seeds were manually sown on plots (2 m wide × 3 m long) on 5 May 2013 using row spacing of 50 cm at a plant density of 10 plants m^{-2} (5 plants m^{-1}). 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. To avoid the water stress, the plots were irrigated twice during the experiment, before seed planting and before flowering stage. Weeds were hand-controlled three times during growing period i.e. on 17 May, 5 June, and 6 July 2013. Moreover, no pesticide was used in the experiment because the crop was not affected by pests or diseases.

Plant height was measured in cm from the ground level to the top of the main stem at pod harvesting stages (72 and 99 days after sowing). Pod length (cm) was recorded on 10 mature pods sampled at random in each plot. Pod number per plant was determined from ten randomly selected plants in each plot. In each plot, pods were harvested from 2 m^2 (two center rows) on 16 July and 12 August 2013. At first pod harvesting stage, third leaves from the top of five randomly selected plants were dried at 72^{o}C for 96 h, and were grounded to pass through a 1-mm sieve and then N and P were measured. N concentration was determined using micro-kjeldahl method following Salicylic-H_2SO_4 digestion (Yamakawa, 1993), and P concentration was determined using the spectrophotometric method of Lowry and Lopez (1946).

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})
1.56	7.9	31.2	51.6	17.2	Silt loam	0.62	0.10	8.0	141

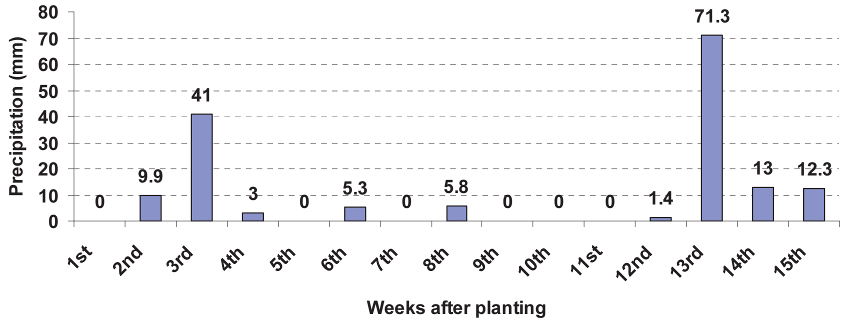

Figure 1. Weekly precipitation during green bean growing period

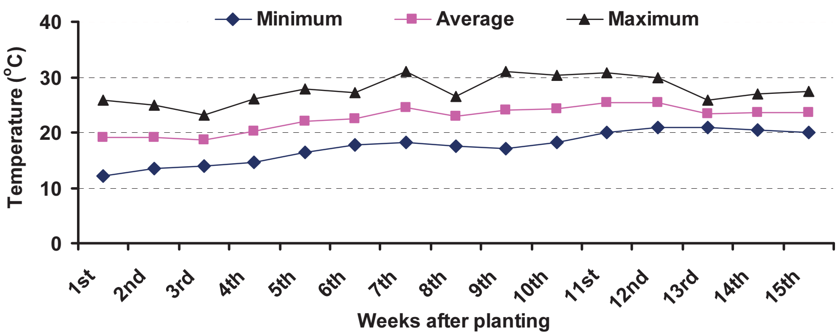

Figure 2. Weekly temperatures (maximum, minimum and average) during green bean growing period

Statistical analyses

Data were subjected to analysis of variance by using PROC GLM of SAS (SAS Inst., 2004). For P rate, where the F-ratios were found to be significant, means separations were conducted using Fisher's protected LSD at the 5% probability level. For Fe foliar application rate, 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 Fe application rate and dependent variables such as pod number per plant and pod yield.

Results and discussion

Plant height

Analysis of variance showed that plant height at 72 and 99 days after sowing (first and final harvesting stages, respectively) were not significantly affected by P rate and iron foliar application rate. Moreover, the interaction between P rate and Fe foliar application rate was not significant (Tables 2 - 4). Similarly, Turuko and Mohammed (2014) reported that P rate had no significant effect on plant height in common bean. In contrast, Shahid et al. (2009) reported that soybean plant height was significantly increased with increasing P application rate. Moreover, Moniruzzaman et al. (2008) noted that plant height of French bean was significantly increased up to 80 kg P_2O_5 ha^{-1} and then remained constant. The promotion effect of P fertilization on corn plant height was attributed to better development of root system and nutrient absorption (Hussain et al., 2006). At the same time, Caliskan et al. (2008) reported that plant height of soybean was significantly increased with increasing Fe dose.

Pod length

The main effects of P rate and Fe foliar application rate were not significant for pod length at both first and second harvesting stages. Moreover, the interaction effect of P rate × Fe foliar application rate was not significant (Table 2 - 4). Our result is inconsistent with Moniruzzaman et al. (2008), who reported that pod length in French bean was significantly increased as P application rate increased from 0 to 80 kg P_2O_5 ha^{-1}, and remained relatively constant at higher P rates (120 kg P_2O_5 ha^{-1}).

Pod number per plant

ANOVA showed that there were significant P rate and Fe foliar application rate effects on pod number per plant at first (PN1) and second (PN2) harvesting stages. At the same time, the interaction effect of P rate × Fe foliar application rate was significant only for PN1 (Table 2). PN1 was significantly increased from 8.4 to 14.5 pods per plant (increased by 72%) as P application rate increased from 0 to 100 kg ha^{-1} (Table 3). PN2 was significantly increased from 23.5 to 39.3 pods per plant (increased by 67%) as P application rate increased from 0 to 100 kg ha^{-1} (Table 3). These results agree with findings of Turuko and Mohammed (2014), who reported that the highest pod number per plant in common bean was recorded with P rates of 20 kg ha^{-1}. Similarly, Moniruzzaman et al. (2008) reported that number of pods per plant in French bean was increased up to 120 kg P_2O_5 ha^{-1} in 2005-2006 and up to 80 kg P_2O_5 ha^{-1} in 2006-2007 growing seasons. This may be due to the fact that P increases flower formation and improves fruit setting. The relationship between Fe foliar application rate and PN1 was well fitted by a linear equation ($Y= 1.49x + 8.09$, $R^2= 0.96$, $P < 0.01$). At the first pod harvesting stage, plants sprayed with 4 and 3 g L^{-1} Fe had higher pod number per plant compared to the others (Figure 3). On the other hand, a quadratic equation ($Y= -0.83x^2 + 4.88x + 27.63$, $R^2=0.91$, $P < 0.05$) provided a good description of the relationship between PN2 and Fe application rate. At the second pod harvesting stage, plants sprayed with 2 g L^{-1} Fe had

Table 2. Mean squares of ANOVA for plant height at 72 (H_{72}) and 99 (H_{99}) days after sowing, pod length at first (PL1) and second (PL2) harvesting stage, pod number per plant at first (PN1) and second (PN2) harvesting stage as affected by phosphorus rate and iron foliar application rate

Source of Variance	df	H_{72}	H_{99}	PL1	PL2	PN1	PN2
Replicate	2	43.1 ns	318 *	1.59 ns	1.71 ns	13 ns	6 ns
Phosphorus rate (P)	2	8.0 ns	139ns	1.34 ns	1.61 ns	145**	937**
Iron foliar application rate (Fe)	4	19.0ns	131 ns	1.93ns	0.33ns	51**	504**
P × Fe	8	59.2 ns	31 ns	1.25 ns	2.1 ns	15 **	26 ns
Error	28	28.6	94	1.48	2.3	4	51
Coefficient of Variance (%)	-	16.8	21.9	12.4	15.0	19.9	22.5

*, ** represent significance at 0.05 and 0.01probability level, respectively; ns represents no significant difference

Table 3. Plant height at 72 (H_{72}) and 99 (H_{99}) days after sowing, pod length at first (PL1) and second (PL2) harvesting stage, pod number at first (PN1) and second (PN2) harvesting stage, pod yield at first (PY1) and second (PY2) harvesting stage, total pod yield (TPY), and leaf N and P concentrations (LNC and LPC, respectively) response to phosphorus rate

Phosphorus rate ($kg\ ha^{-1}$)	Traits										
	H_{72}	H_{99}	PL1	PL2	PN1	PN2	PY1	PY2	TPY	LNC	LPC
	(cm)				No. $plant^{-1}$		($kg\ ha^{-1}$)			(%)	
0	31.9	41.0	10.0	10.3	8.4	23.5	5223	9568	14791	1.78	0.15
50	31.0	45.0	9.4	9.9	10.2	32.5*	5712	10692	16404*	1.79	0.15
100	32.4	46.9	9.9	10.2	14.5*	39.3*	7018*	12544*	19562*	1.95	0.16
LSD (0.05)	4.0	7.2	0.9	1.1	1.6	5.3	522	1385	1457	0.19	0.02

* indicates significant difference over control

Table 4. Plant height at 72 (H_{72}) and 99 (H_{99}) days after sowing, pod length at first (PL1) and second (PL2) harvesting stage, and leaf N and P concentrations (LNC and LPC, respectively) response to Fe foliar application rate

Fe foliar application rate ($g\ L^{-1}$)	Traits					
	H_{72}	H_{99}	PL1	PL2	LNC	LPC
		(cm)			(%)	
0	30.9	39.8	9.2	10.2	1.45	0.14
1	30.0	42.8	10.3	10.3	2.02*	0.15
2	32.3	49.7	9.4	10.2	1.94*	0.16
3	31.8	42.7	10.0	9.9	1.94*	0.18*
4	33.8	46.5	9.8	10.0	1.87*	0.13
LSD (0.05)	5.1	9.3	1.2	1.5	0.25	0.03

* indicates significant difference over control

higher pod number per plant compared to the others (Figure 3). These results are consistent with those of Caliskan et al. (2008) in soybean, who reported that the numbers of pod per plant significantly increased with Fe foliar application.

Pod yield

The main effects of P rate and Fe foliar application rate were significant for pod yield at first harvesting (PY1), pod yield at second harvesting (PY2), and total pod yield (TPY). At the same time, the interaction between P rate and Fe foliar application rate was significant only for PY1 (Table 5). PY1 was significantly increased with increasing P application rate. The highest (7018 kg ha^{-1}) and the lowest (5223 kg ha^{-1}) pod yields at first harvesting stage were obtained when P was applied at the rate of 100 and 0 kg ha^{-1}, respectively (Table 3). PY2 was significantly increased from 9568 to 12544 kg ha^{-1} as P application rate increased from 0 to 100 kg ha^{-1} (Table 3). Total pod yield was significantly increased by 32% when P application rate increased from 0 to 100 kg ha^{-1} (Table 3). Moniruzzaman et al. (2008) and Srinivas and Naik (1990) reported that significantly highest pod yield was recorded at 80 kg P_2O_5 ha^{-1}. Phosphorus is an essential macronutrient that improves plant growth and yield through the following: 1) P promotes root growth and stimulates lateral root branching that, in turn, increases nutrients absorption from the soil; 2) it plays a vital role in flower formation and fruit setting; 3) it is involved in sugar and starch utilization, photosynthesis, and cell division; 4) it stimulates leaf cell division and elongation, and leaf number, thus increases leaf area index (Assuero et al., 2004; Kavanova´ et al., 2006), and this improves light interception and photosynthesis and, therefore, increases plant biomass accumulation, and 5) it is a crucial element for nodule formation in legume crop and improves nitrogen fixation (Bhuiyan et al., 2008).

The relationship between Fe foliar application rate and PY1 was well expressed by a linear equation ($y=-166.59x^2 + 1479.14x + 4025.71$, $R^2=0.90$, $P < 0.05$). PY1 was significantly increased from

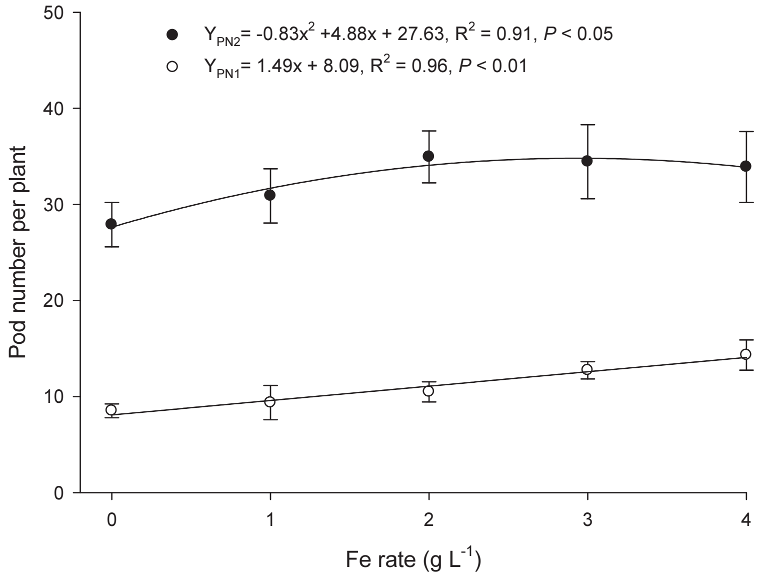

Figure 3. Effect of Fe foliar application rate on pod number per plant at first (PN1) and second (PN2) harvesting stage, when averaged across phosphorous rate. Vertical bars represent ± 1 SE of means

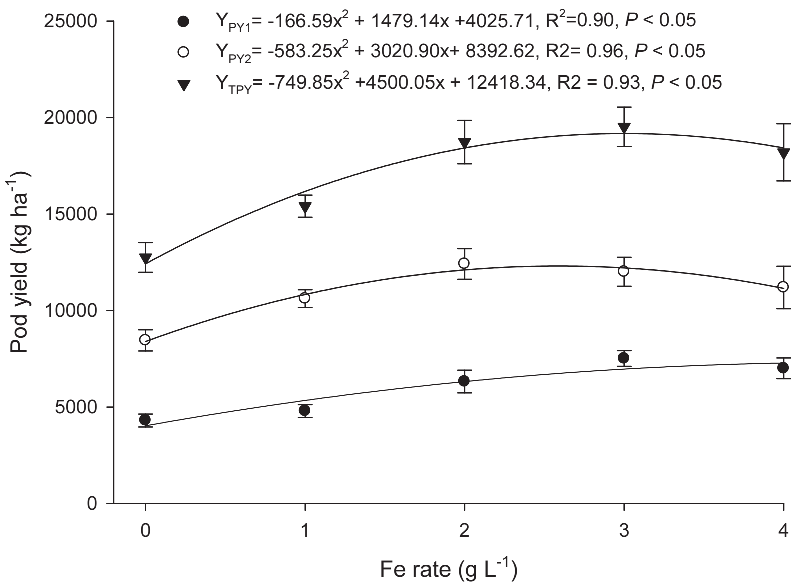

Figure 4. Effect of Fe foliar application rate on pod yield at first (PY1) and second (PY2) harvesting stage, and total pod yield (TPY), when averaged across phosphorous rate. Vertical bars represent ± 1 SE of means

4300 to 7513 kg ha^{-1} as Fe foliar application rate increased from 0 to 3 g L^{-1} and thereafter relatively remained constant (Figure 4). The relationship between P application rate and PY2 was well expressed by a quadratic equation ($y=-583.25x^2 + 3020.90x + 8392.62$, $R^2=0.96$, $P < 0.01$). PY2 was significantly increased by 46% as Fe foliar application rate increased from 0 to 2 g L^{-1} and thereafter slightly decreased (Figure 4). TPY followed similar trend to PY2 ($y=-749.85x^2 + 4500.05x + 12418.34$, $R^2=0.93$), although the relationship between Fe rate and TPY was significant at 0.05 probability level. TPY was significantly increased from 12747 to 18727 kg ha^{-1} as Fe foliar application rate increased from 0 to 3 g L^{-1}and thereafter relatively remained at a stable level (Figure 4). Zaiter et al. (1992) reported that seed yield of dry bean (*Phaseolus vulgaris* L.) was significantly increased when sprayed with Fe. Goos and Johnson (2000) reported that foliar sprays of FeEDTA significantly increased seed yield in soybean. Previous studies indicated that adequate Fe supply increased nitrogenase activity and biological N fixation capacity of soybean (Chonkar and Chandel, 1991; Terry and Jolley, 1994). These results can be attributed to the fact that Fe acts an essential element for mineral nutrition of rhizobia bacteria, nodulation, nodule activity and biological N fixation (O'Hara, 2001). Apart from the effect of high pH on decreasing Fe uptake by the plants, the impaired effect of high soil pH on growth and nodulation was reported by Tang et al. (1991) in peanut and Tang and Robson (1993) and Tang et al. (2006) in lupinus species. Similarly, Singh et al. (1990) found that foliar spray of Fe-EDTA increased significantly pod yield of groundnut.

Leaf N concentration

The main effect of Fe foliar application rate was significant ($P < 0.01$) for leaf N concentration, while the main effect of P rate was not significant (Tables 3-5). Moreover, the Fe foliar application rate × P rate had significant effect on leaf N concentration ($P < 0.01$). The highest leaf N concentration was observed for plot receiving 50 kg P ha^{-1} and spraying with 2 g L^{-1} Fe, while

Table 5. Mean squares of ANOVA for pod yield at first (PY1) and second (PY2) harvesting stage, total pod yield (TPY), and leaf N and P concentrations (LNC and LPC, respectively) as affected by phosphorus rate and iron foliar application rate

Source of Variance	df	PY1	PY2	TPY	LNC	LPC
Replicate	2	10231635 **	7931748 ns	28856606**	0.16ns	0.007ns
Phosphorus rate (P)	2	12913184**	3387221**	88327146**	0.13ns	0.0077 ns
Iron foliar application rate (Fe)	4	17432538**	2178258**	70537236**	0.45**	0.002*
P × Fe	8	1677357 **	3793254 ns	6274738 ns	0.50 **	0.003 **
Error	28	488018	3432622	3795847	0.06	0.0007
Coefficient of Variance (%)	-	11.6	16.9	11.5	14.2	17.7

*, ** represent significance at 0.05 and 0.01probability level, respectively; ns represents no significant difference

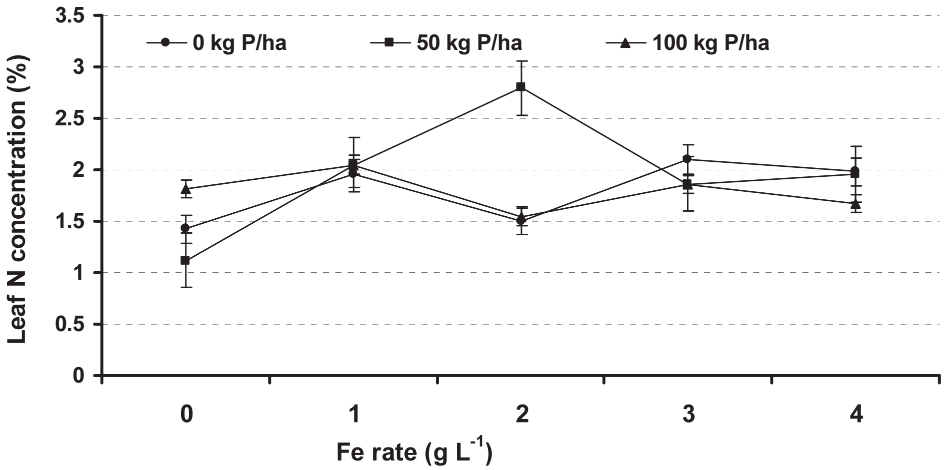

Figure 5. Fe foliar application rate × P rate interaction effect on leaf N concentration. Vertical bars represent ± 1 SE of means.

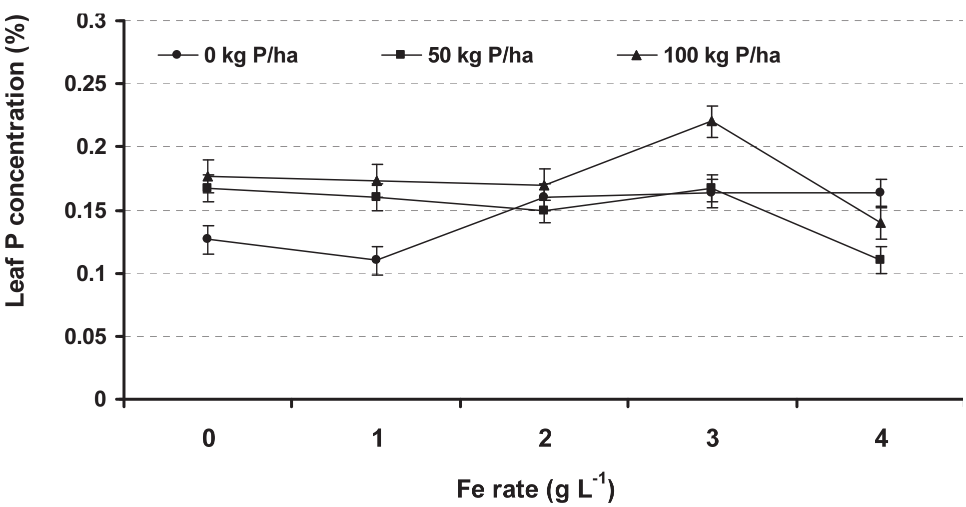

Figure 6. Fe foliar application rate × P rate interaction effect on leaf P concentration. Vertical bars represent ± 1 SE of means

the lowest ones were observed for plots received 50 kg P and Fe 0 or for plots received neither P nor Fe (Figure 5). This result is in agreement with that of Singh et al. (1990), who reported that foliar application of Fe-EDTA increased the leaf N concentration in groundnut. ANOVA (Table 5) also showed that the main effect of P rate was non-significant ($P > 0.05$). Contrary to this result, Dwivedi and Bapat (1998) reported that nitrogen content in soybean increased significantly by P application up to 50 kg ha^{-1}. It has been demonstrated that phosphorus and nitrogen are closely related in crop nutrition. Li and Zhao (1990) found that the supply of phosphorus improved nitrogen metabolism in plant. At the same time, the supply of nitrogen is necessary to allow crops to use phosphorus (Li and Zhao, 1990).

Leaf P concentration

The main effect of Fe foliar application rate and the interaction between Fe foliar application rate and P rate were significant for leaf P concentration (Tables 3-5). The highest leaf P concentration was observed for plot receiving 100 kg P ha^{-1} and spraying with 3 g L^{-1} Fe, while the lowest ones were observed for plots receiving no P fertilizer and spraying with 0 or 1 g L^{-1} Fe or for plots receiving 50 kg P fertilizer and spraying with 4 g L^{-1} Fe (Figure 6). Contrary to this result, Edral et al. (2004) reported that foliar Fe applications did not significantly affect leaf P concentrations. ANOVA also indicated that the main effect of P rate was not significant for leaf P concentration (Tables 3-5). These results are inconsistent with those of Singh and Singh (2004) and Chandra and Khaldelwal (2009), who reported that adequate P supply increased significantly P content in grain and straw of black gram (*Phaseolus mungo* L.) and chickpea (*Cicer arietinum* L.), respectively.

Conclusions

This experiment illustrated that pod yield was significantly increased by 32% when P application rate increased from 0 to 100 kg ha^{-1}. At the same time, pod yield was significantly increased by 46% as Fe foliar application rate increased from 0 to 2 g L^{-1} and thereafter relatively remained constant. Based on the results of this experiment, 100 kg ha^{-1} P application and 2 g L^{-1} Fe foliar application were the most consistent treatments for achieving the highest pod yield in green bean.

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