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Effect of *Azotobacter* and Chemical Phosphorus Fertilizer on Maize.

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ABSTRACT

To study the effects of phosphorus and *Azotobacter* on yield of maize, a factorial experiment was carried out based on randomized complete block design with three replications in Astara, north of Iran, in 2012. The treatments were phosphorus fertilizer rates (0, 62.5, 125, 187.5 and 250 kg P_2O_5 ha^{-1}) and seed treatment (inoculated with *Azotobacter Vinelandii* and uninoculated). The analysis of variance indicated that phosphorus fertilizer improved significantly grain yield. Because of non significant effect of two factors, the average of data across two conditions of inoculated and uninoculated with *Azotobacter Vinelandii* was used for drawing the graph. For nitrogen content in grain the interaction effects of two factor was significant and the graph drawn separately. The effects of *Azotobacter* were significant on nitrogen concentrations in grain and ear length. Phosphorus at the rate of 187.5 kg ha^{-1} was the optimum rate to cause a desirable increase in grain yield, thousand grain weights, biological yield, leaf area index, nitrogen content in grain, grain P uptake and ear length. Overall, utilization of biological nitrogen with chemical phosphorus fertilizer could be a strategy to achieve sustainable agriculture.

Keywords: Corn, Nitrogen fixation, *Azotobacter*, Uptake, Yield component.

INTRODUCTION

Maize (*Zea mays L.*) is an important field crop and is the third most widely cultivated cereal crop after wheat and rice in world. It is grown over large areas because it is used as a source for food for human and also as a fodder. Maize grown for green fodder harvested 8-10 weeks after sowing (Hameeda *et al.,* 2008).

The grain yield of maize depends on the genetic potential of a hybrid, soil characteristics, agrotechnical applications and climatic conditions (Jockovic *et al.,* 2010). In conventional agriculture large amounts of mineral fertilizers are applied which increase cost of production and may be harmful to environment while, organic production includes only the use of organic fertilizers and biofertilizers (Dai *et al.,* 2004). Biofertilizers are microbiological fertilizers that include specific species of microorganisms and used for stimulating microbiological processes in which plant nutrients are released (Rodriguez *et al.,* 2004). *Azotobacter* is a beneficial free living (non symbiosis) nitrogen fixing bacteria which is reported to fix 20-60 kg N ha^{-1}. It has been widely used to inoculate crops and results indicated that seed inoculation of non-legume increased the yield of field crops by about 10% and cereals by 15- 20% (Forlain *et al.,* 1998). Several mechanisms have been suggested for promoting plant growth by Azotobacter including phytohormone production, enhancing stress resistance, N_2 fixation, stimulation of nutrient uptake and biocontrol of

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pathogenic microorganisms (Rodriguez and Fraga, 1999), increasing the supply or availability of primary nutrients to the host plant (Wu *et al.,* 2005).

Naseri *et al.,* (2013) indicated that there was significant effect of Azotobacter on plant height, number of grain per row, 1000-grain weight, grain yield, biological yield and protein content. Pandey *et al.*, (1998) mentioned that introducing *Azotobacter chroococcum* and *Azospirillum brasilense* into soil under maize stimulated the growth of actinomycetes and free-living nitrogen-fixers. Golami *et al*., (2009) reported that grains number increased with seed priming with *Azotobacter* in maize. Hajnal-Jafari *et al.,* (2012) indicated the grain yield increased with inoculation by *Azotobacter*. Meshram and Shende (1982) declared *Azotobacter* inoculation was economically most efficient at lower doses of nitrogen which not only increased yields but resulted in a saving of N when applied in combination with farmyard manure.

Phosphorus (P) is the most important nutrient element (after nitrogen) limiting agricultural production in most regions of the world (Kogbe and Adediran, 2003). The effects of chemical phosphates were studied on maize by some researchers. Maqsood *et al*., (2001) showed phosphorus significantly affected maize plant height, number of cobs per plant, number of grains per cob and grain yield. They also deduced P should be applied at the rate of 100 kg ha^{-1} for best grain yield. Ali *et al*., (2002) reported significant effect of P on grain yield; whereas Ayub et al (2002) indicated significant effect of P on dry matter yield and plant height, number of leaves and leaf area. Rashid and Iqbal (2012) indicated maize fodder yield was increased significantly with application of phosphorus. Onasanya *et al.,* (2009) concluded application rate of 120 kg N ha^{-1} + 40 kg P ha^{-1} may be recommended for increasing maize yield.

The objective of this research was to study the effect of phosphorus fertilizer and *Azotobacter Vinelandii* on grain yield and yield components of maize.

METHODOLOGY

This experiment was conducted in 2012, in Iran, Astara region (longitude, 48° 51´ E; latitude, 38° 267´ N; altitude, 15 m above sea level). The experimental was a factorial, randomized complete block design with three replications having a plot size of 4.50 m × 4.0 m with distance of 0.75 m between rows and 0.20 m between plants. Soil properties are presented in Table 1.

All plots received urea (50 kg ha^{-1}) and KCl (100 kg ha^{-1}) as a basal application. Subsequent dressings of N fertilizer at a rate of 25 kg ha^{-1} as urea were applied at 3 weeks after emergence and again at silking stage. The phosphorus fertilizer rates were 0, 62.5, 125, 187.5 and 250 kg P_2O_5 ha^{-1} applied as triple super phosphate and *Azotobacter* as nitrogen fixation bacteria (uninoculation and inoculation with *Azotobacter Vinelandii*). Inoculation was performed with strain04 of *Azotobacter Vinelandii*, with concentration of 10^9 per ml. Seed inoculation was performed before sowing at a rate of 7 ml kg^{-1}.

All recommended agronomic practices were carried out throughout the growing season. The weeds were controlled with hand. The parameters studied during the experiment were plant height, grain yield, total grain number per ear, thousand grain weights, biological yield, harvest index, ear length, leaf area index, grain N concentration, grain N uptake, grain P concentration and grain P uptake. Plants that harvested from each plot after drying in an Awan set as 75 c for 48h were weighed and biological yield per ha was measured. The percentage of nitrogen concentrations in grain were measured by Kjeldahl method and the percentage of phosphorus concentrations in grain, in the samples digested in sulfuric acid and hydrogen peroxide, by colorimetric analysis using the phosphovanadomolybdic complex. Grain N uptake was calculated as following equation:

Grain N uptake (kg ha^{-1}) = Grain N concentration (%) * Grain yield (kg ha^{-1})

Grain P uptake was also calculated as following equation:

Grain P uptake (kg ha^{-1}) = Grain P concentration (%) * Grain yield (kg ha^{-1})

Data were analyzed by SAS Ver.9 program. Analysis of variance (ANOVA) procedures were run for all of the traits. Mean separation was done using least significance difference (LSD) test to signify the treatment differences at 5% level of probability.

Graphs and Nonlinear regression equations were derived using Excel. Visual traits were regressed against P rate using linear and nonlinear equations. The corrected R^2 for all linear and nonlinear regressions were calculated by subtracting the ratio of the residual sum of squares to the corrected total sum of squares from one. Statically, because of non significant effect of two factors, the data must be averaged across Azotobacter inoculated and uninoculated conditions. Therefore, the average of data across two conditions was used for drawing the graph. For Figure 5 (nitrogen content in grain) the interaction effects of two factor was significant and the graph drawn separately.

Table 1. Some of the soil characteristics.

Sampling Depth (cm)	0-30
Clay(%)	35
Silt (%)	44
Sand(%)	21
Textural Class	Loam/Clay
Electrical conductivity (ds/m)	0.89
Saturation Percentage (SP)	54
pH	7.1
Soil Organic Carbon(%)	2.11
Total Nitrogen(%)	0.19
Available Phosphorus (mg/kg)	7.88 (Low)
Available Potassium (mg/kg)	287 (Sufficient)

RESULTS

The analysis of variance indicated the effects of phosphorus fertilizer were significant on grain yield, thousand grain weight, biological yield, leaf area index, nitrogen content in grain, grain nitrogen uptake, phosphorus content in grain, grain phosphorus uptake and ear length. The effects of *Azotobacter* were significant on nitrogen content in grain, grain nitrogen uptake and ear length. Interaction effects of two factors were significant only on nitrogen content in grain (Table 2).

Table 2. Analysis of variance for some of traits in corn as affected by phsphorus fertilizer rate and Azotobacter.

S.O.V	df	Mean-squares					
		Plant height (cm)	Grain yield (kg ha^{-1})	Total grain number per plant	Thousand grain weight (g)	Biological yield (kg ha^{-1})	Harvest index
Replication	2	203.54	7309009.60**	5682.35	4082.00 **	12827857.6	51.05
Phosphorus fertilizer	4	322.26	5084880.45**	12803.75	2368.25 *	33373790.3 **	16.30
Azotobacter Vinelandii	1	0.12	789265.20	362.26	267.00	5151820.8	0.019

S.O.V	df	Mean-squares					
		Plant height (cm)	Grain yield (kg ha^{-1})	Total grain number per plant	Thousand grain weight (g)	Biological yield (kg ha^{-1})	Harvest index
***Azotobacter Vinelandii* * Phosphorus fertilizer**	4	47.45	30865.95	61.57	4.32	172469.6	0.48
Error	18	191.82	462138.49	6538.78	626.77	3848268.3	29.11
CV		7.87	8.74	16.94	10.69	9.59	14.10

S.O.V	df	Mean-squares					
		LAI: Leaf area index	Nitrogen content in grain (%)	Grain nitrogen uptake (kg ha^{-1})	Phosphorus content in grain (%)	Grain phosphorus uptake (kg ha^{-1})	Ear length (cm)
Replication	2	0.63^{**}	0.021^{**}	3568.75^{**}	0.00123	90.09^{**}	2.25^{**}
Phosphorus fertilizer	4	1.19^{**}	0.116^{**}	4118.35^{**}	0.00943^{**}	177.71^{**}	3.74^{**}
Azotobacter Vinelandii	1	0.022	1.82^{**}	15251.91^{**}	0.00019	9.89	1.36^{**}
***Azotobacter Vinelandii* * Phosphorus fertilizer**	4	0.017	0.045^{**}	372.90	0.000006	0.039	0.041^{ns}
Error	18	0.085	0.003	151.70	0.00037	4.63	0.047
CV		8.77	3.10	8.82	7.54	10.63	1.19

**: r significant at the 1% probability levels

Grain yield

The analysis of variance indicated significant effect of phosphorus fertilizer on grain yield (Table 2). It seems that phosphorus plays an important role in enhancement of grain yield. The application of chemical phosphorus fertilizer had significant effect to increase the yield of maize. Grain yield followed a positive linear equation as P_2O_5 rate increased from 0 to 250 kg ha^{-1}. In general, the maximum grain yield (8835 kg ha^{-1}) was obtained by use of phosphorus at rate of 250 kg P_2O_5 ha^{-1}, while the least value (6677 kg ha^{-1}) was recorded in control plots (Figure 1). The results indicated that there was not significant difference between rates of 187.5 and 250 kg P_2O_5 ha^{-1}.

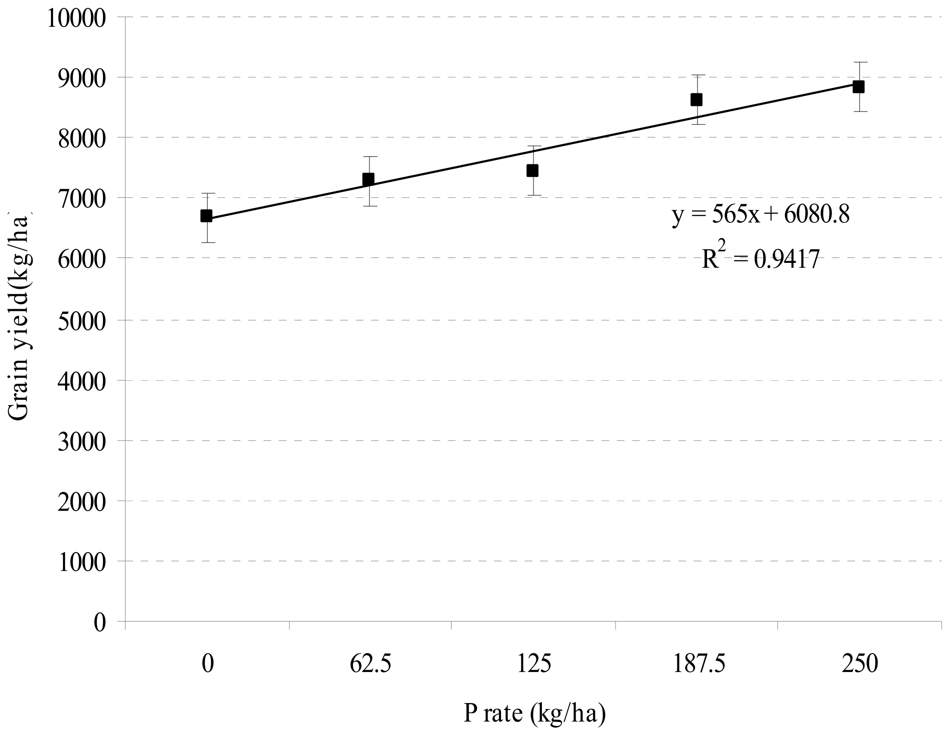

Figure 1. Effect of phosphorus fertilizer rate on grain yield. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

Thousand grain weight

Thousand grain weight was significantly affected by phosphorus fertilizer. Maximum value of thousand grain weight was recorded with use of phosphorus at rate of 250 kg ha^{-1} (253.13 g) and minimum value of it was recorded at control (206.25 g). The results indicated thousand grain weight followed a positive quadratic relationship as P rate increased from 0 to 250 kg ha^{-1} (Figure 2). The results indicated that there was no significant difference between thousand grain weight at the rate of 125, 187.5 and 250 kg phosphorus ha^{-1}.

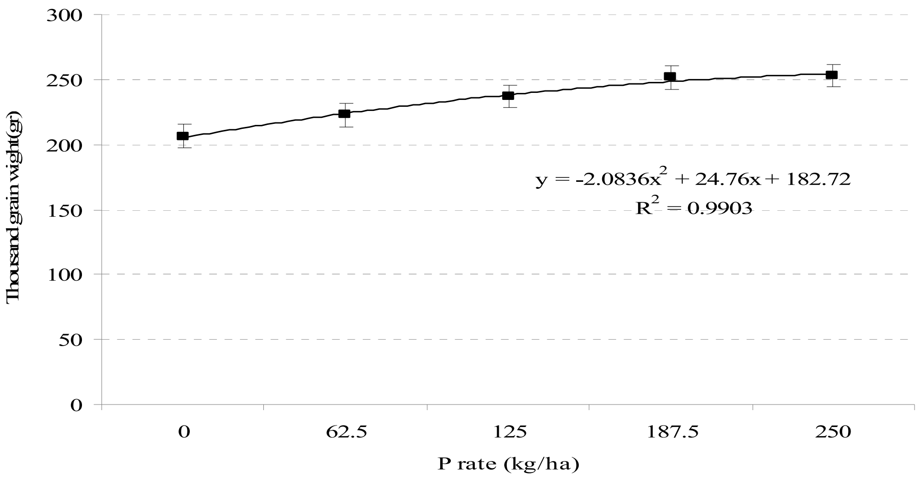

Figure 2. Effect of phosphorus rate on thousand grain weight. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

Biological yield

The results indicate that the highest value of biological yield (22646 kg ha^{-1}) was obtained by utilization of phosphorus fertilizer at the rate of 250 and the lowest value of it was recorded in control (16974 kg ha^{-1}). There was no significant difference between phosphorus rates above 125 kg ha^{-1} and the quadratic equation indicate that biological yield increased as the rate of phosphorus increased up to 125 kg ha^{-1}, but did not increase significantly with further increase in the phosphorus rate. It is concluded that P_2O_5 should be applied at the rate of 125 kg ha^{-1} for best biological yield (Figure 3).

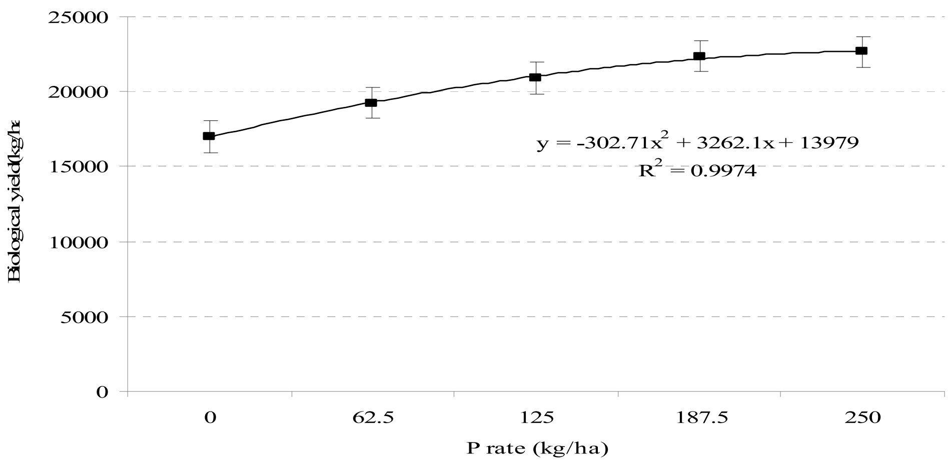

Figure 3. Effect of phosphorus rate on biological yield. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

Leaf area index

The results indicate that Leaf area index was significantly affected ($P \leq 0.05$) by phosphorus fertilizer rate (Table 2). Mean values of the data indicated that maximum LAI (3.75) was recorded in plots with P applied at the rate of 250 kg ha^{-1} followed by P_2O_5 at the rate of 187.5 kg ha^{-1} (3.68). There was no significant difference between 125, 187.5 and 250 kg P ha^{-1} rates (Figure 4).

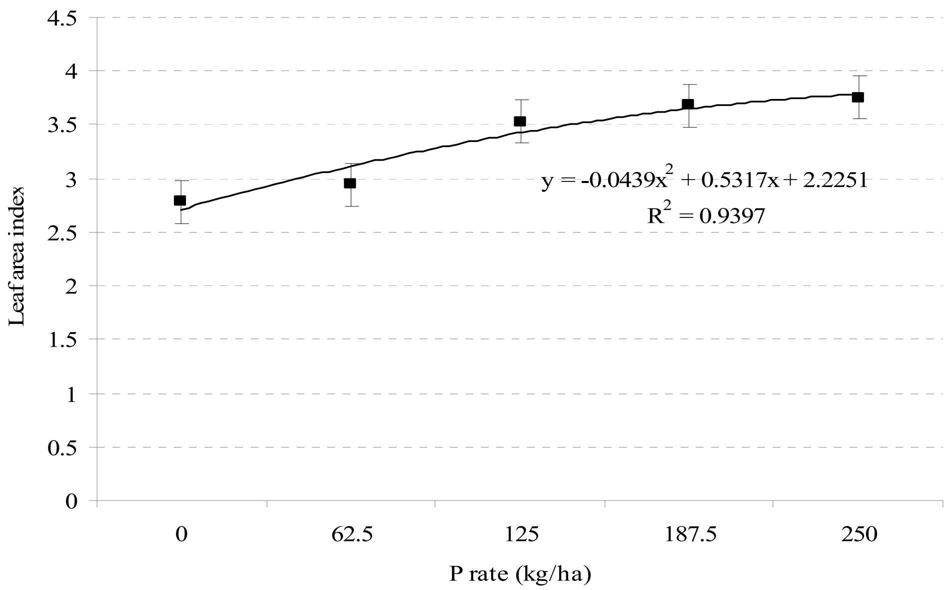

Figure 4. Effect of phosphorus rate on leaf area index. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

Nitrogen content in grain and grain nitrogen uptake

The interaction effect of two factors was significant on nitrogen concentration in grain. A linear equation explained the relationship between phosphorus fertilizer rate and grain N concentration in noninoculation condition (Figure 5). In this condition, grain N concentration increased slowly with P application rate. In contrast, in inoculation condition with *Azotobacter*, a quadratic equation expressed the relationship between phosphorus fertilizer rate and grain N concentration. Grain N concentration increased significantly as P_2O_5 application rate increased from 0 to 125 kg ha^{-1}, but there was no significant difference between 125 kg ha^{-1} and the above rates (Figure 5).

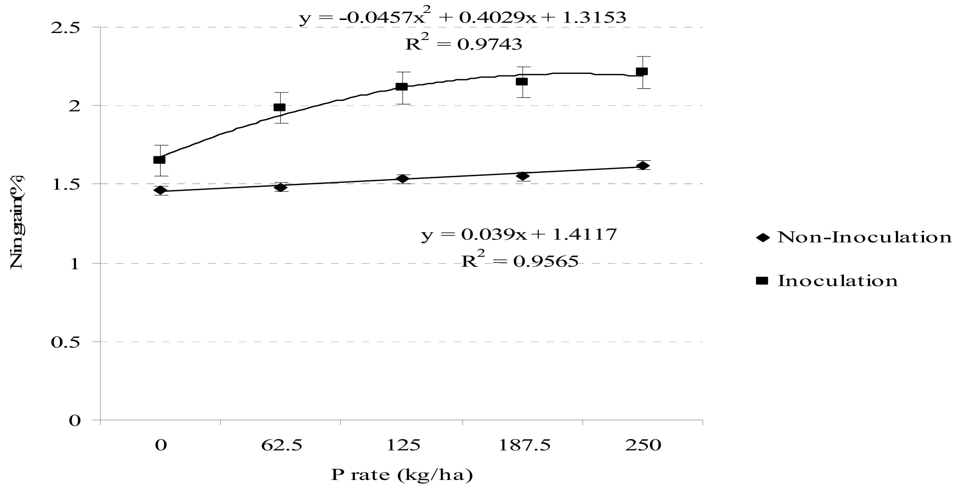

Figure 5. Effect of phosphorus fertilizer rate on nitrogen content in grain in inoculated and uninoculated plants. Vertical bars represent means ± 1 standard error.

Maximum grain N uptake (162.05 kg ha^{-1}) was recorded when seeds were inoculated with *Azotobacter*. The results indicated that the lowest value of grain N uptake (104.09 kg ha^{-1}) was recorded when phosphorus fertilizer was not applied. Maximum value of grain N uptake (169.77) was recorded when phosphorus fertilizer was applied at the rate of 250 kg ha^{-1}. As expected, there was no significant difference between 187.5 and 250 kg P_2O_5 ha^{-1} rates (Figure 6). Nitrogen uptake varied from 104.1 kg N ha^{-1} in the control plot to 169.78 kg N ha^{-1} in the plots treated with phosphorus at rate of 250 kg ha^{-1}. There were not significant differences between 187.5 and 250 kg P_2O_5 ha^{-1} (Figure 6).

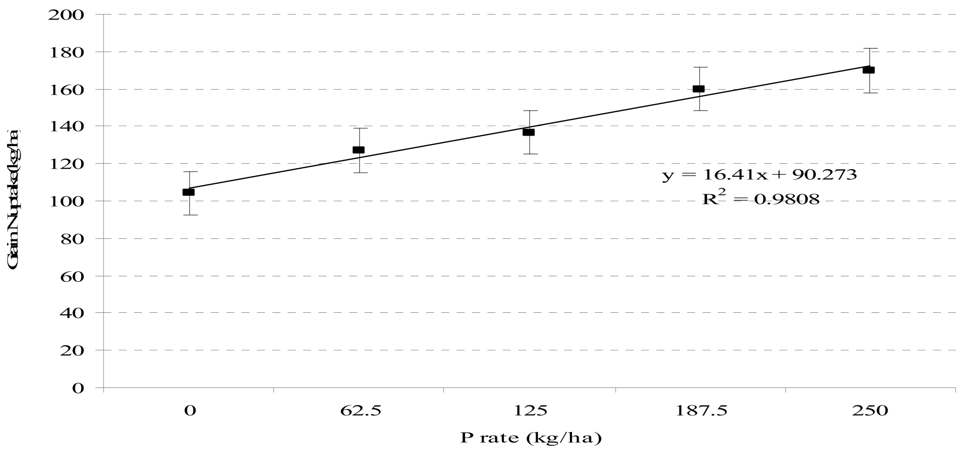

Figure 6. Effect of phosphorus rate on grain N uptake. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

Phosphorus content in grain and grain phosphorus uptake

Grain P concentration followed a positive quadratic relationship as P rate increased from 0 to 250 kg ha^{-1}. The lowest value (0.21 %) of grain P concentration was observed in control plots. The highest grain P concentration (0.31 %) was recorded of 250 kg P_2O_5 ha^{-1} rate; this value was significantly higher than the values recorded in other P rates (Figure 7).

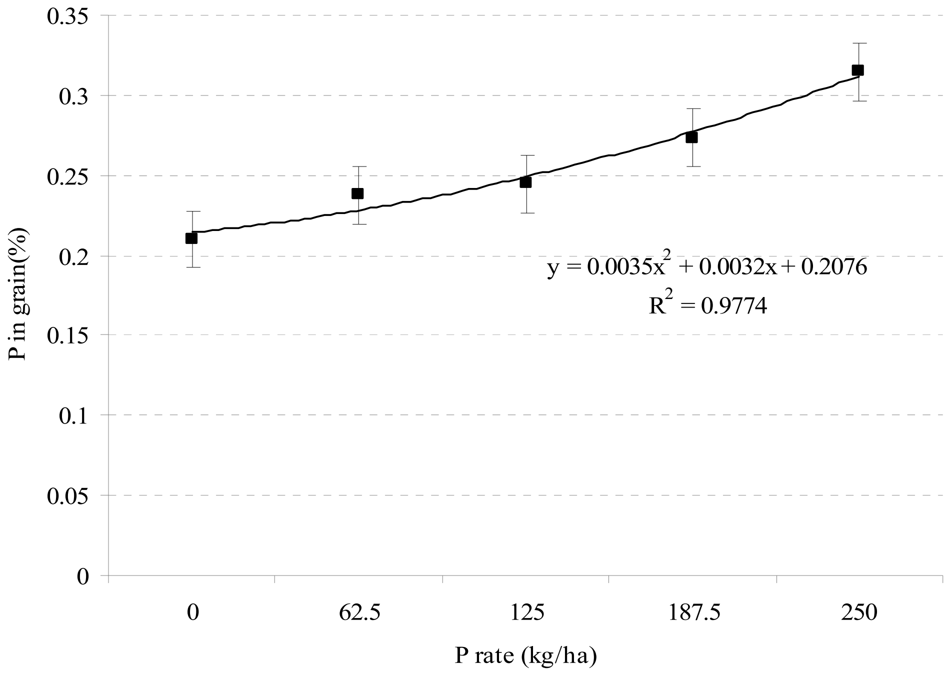

Figure 7. Effect of phosphorus rate on grain P concentration. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

There was significant P uptake response to the phosphorus fertilizers (Table 2). The P uptake which expressed by a quadratic equation responded to phosphorus fertilizer rate, varied with the fertilizers from 14.17 kg ha^{-1} P in the control plots to 27.80 kg ha^{-1} in plots with 250 kg P_2O_5 ha^{-1}. There was no significant difference in grain P uptake between 187.5 and 250 kg P_2O_5 ha^{-1} rates (Figure 8).

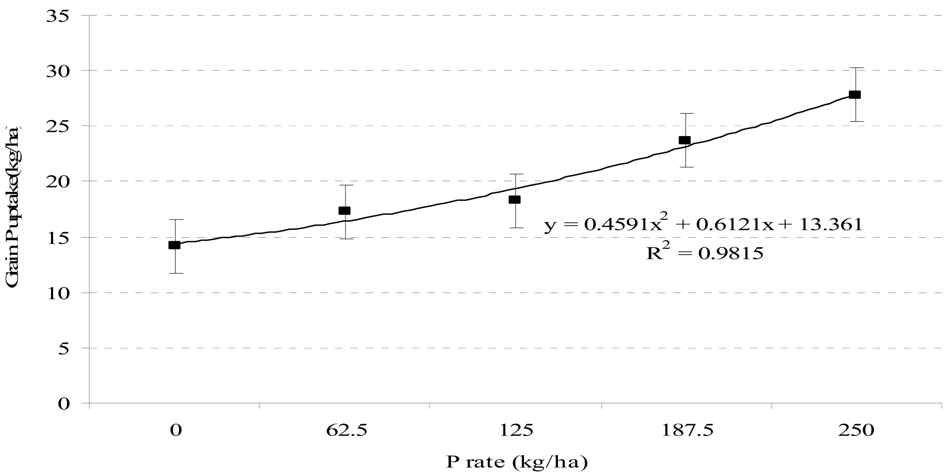

Figure 8. Effect of phosphorus rate on grain P uptake. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

Ear length

The response of ear length to *Azotobacter* and phosphorus fertilizer was significant but response of it to interaction of two factors was not significant. Maximum (18.47 cm) and minimum ear length were (18.05 cm) recorded with *Azotobacter* inoculation and non-inoculation, respectively. The response of ear length to phosphorus fertilizer indicated that maximum ear length (19.13 cm) was observed from 250 kg P_2O_5 ha^{-1}. The relation between ear length and phosphorus rate was according a linier equation and there were not significant differences between 187.5 and 250 kg P_2O_5 ha^{-1} (Figure 9).

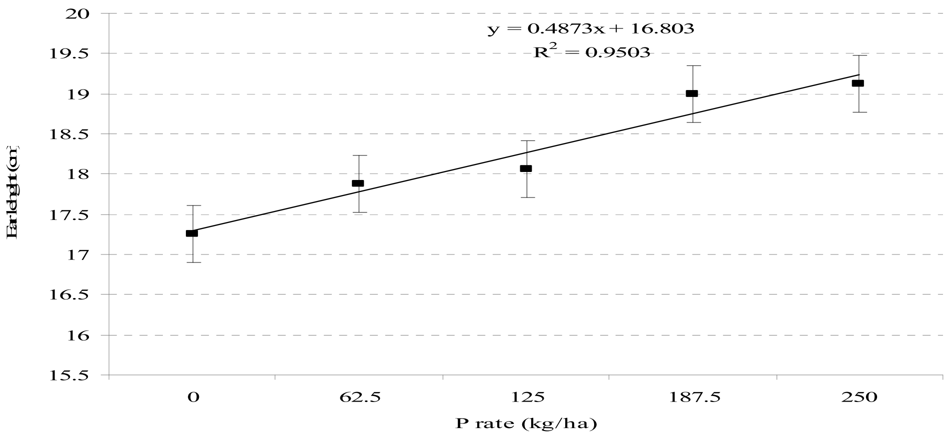

Figure 9. Effect of phosphorus rate on ear length. (According to no significant effect of two factor interactions, the data was averaged across *Azotobacter* inoculated and uninoculated and used for drawing the graph). Vertical bars represent means ± 1 standard error.

DISCUSSION

The results of this experiment were indicated that applying P_2O_5 at the rate of 187.5 kg ha^{-1} might be the optimum rate to cause a desirable increase in grain yield, thousand grain weight, biological yield, leaf area index, nitrogen content in grain, grain P uptake and ear length, because the grain yield and the other traits in the control plots was the lowest whereas it was highest in the plots with P_2O_5 applied at 187.5 kg ha^{-1}. Therefore, further increase in P_2O_5 above 187.5 kg ha^{-1} did not have a directly proportional effect on the mentioned traits of maize, which is obvious from the plots with P_2O_5 application at the rate of 250 kg ha^{-1}. Increasing P_2O_5 above 187.5 kg ha^{-1} might be excessive that had not increased the grain yield of maize which indicated that applying P_2O_5 in maize above 187.5 kg ha^{-1} is uneconomical and just wastage of money. The results of this experiment were similar to the finding of Maqsood *et al.*, (2001), Ali *et al.*, (2002), Ayub *et al.*, (2002), Rashid and Iqbal (2012) and Onasanya *et al.*, (2009) who indicated significant effect of P on maize grain yield. Data regarding to phosphorus showed that application of phosphorus fertilizer increased its concentration and uptake in maize. The results are in agreement with the findings of Chaudhary *et al.*, (2003) who observed that phosphorus contents in maize fodder significantly increased with increasing soil solution phosphorus levels in all the soil series.

In this study, it was also observed that inoculation maize of seed with *Azotobacter*, compared to uninoculated seeds, increased the nitrogen content in grain, grain nitrogen uptake and ear length. The comparison of these traits in two inoculation conditions indicated the positive effect of *Azotobacter* to increase the nitrogen concentrations and its uptake in the maize plant. The positive effects of *Azotobacter* inoculation on these traits in 100 kg N ha^{-1} fertilized applying condition shows that there is a possibility for mineral nitrogen to be partially replaced by biological nitrogen and are in agreement with Hussain *et al.* (1987) who concluded that *Azotobacter* increased the maize grain yield and N concentration in grain in the presence of fertilizers i.e, NPK ~ 125, 125, 125 kg ha^{-1}, respectively. Quansah (2010) indicated the combined treatments of biofertilizer and fertilizer promoted significantly higher N and P uptake in both the grain and stover in maize than the sole organic or inorganic treatments. This may be due to the fact that the combined treatments improved the soil environment which was efficiently exploited by the maize plants as compared to the sole organic or inorganic treatments. This result also is consistent with data of Jarak *et al.,* (2011) who arrived at similar conclusions concerning the use of free-living and associative nitrogen-fixing bacteria in maize production. Moreover, Shaukat *et al.,* (2006) and Egamberdiyea (2007) stated that *Azotobacter* biofertilizer increase content of nitrogen by stimulating processes such as seed germination, resistance of seedlings to stress conditions, nitrogen fixation and production of phytohormones.

Table 3. Mean comparison of some traits influenced by *Azotobacter*.

Azotobacter Vinelandii	**Grain nitrogen uptake (kg ha^{-1})**	**Ear length (cm)**
Non-inoculation	116.95^{b}	18.05^{b}
Inoculation	162.05^{a}	18.47^{a}

Members followed by the same letter are not significantly different at 0.05 probability level.

CONCLUSIONS

This experiment documented that P fertilizer had significant positive effect on seed yield and yield components of maize. Phosphorus at the rate of 187.5 kg ha^{-1} has the best performance in obtaining maximum grain yield, thousand grain weight, biological yield, leaf area index, nitrogen content in grain, grain N uptake and ear length of maize. This study showed also that application of *Azotobacter* significantly increased nitrogen content in grain, grain nitrogen uptake and ear length. Overall, utilization of biological nitrogen fixation fertilizer with chemical phosphorus fertilizer and 100 kg N ha^{-1}, in addition to increased nitrogen content as a protein resource could be a strategy to achieve sustainable agriculture by partially replacement of mineral nitrogen with biological nitrogen.

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تأثير الآزوتوباكتر والفوسفور الكيميائي على الذرة

باريا هاشمنيا [1] وبيجن شريفي [2]✉ وهاشم أمينبان [1]

ملخص

لدراسة تأثير الفوسفور والباكتريا المثبته للنايتروجين على محصول الذرة، أجريت في عام 2012. تجربة عاملية على أساس تصميم القطاعات العشوائية الكاملة في ثلاثة مكررات في مدينة أستارا، شمال إيران وكانت المعاملات هي معدلات الفوسفور والأسمدة (0، 62/5 ، 125، 187/5 و 250 كيلوغرام P_2O_5 في الهكتار الواحد)، وتلقيح البذور بالبكتريا *Azotobacter Vinelandii* (تلقيح وبدون التلقيح). أشارت نتائج التحليل الإحصائي إلى أن تأثير معاملات التسميد بالفوسفور كانت معنوية على جميع الصفات المدروسة. نظرا لعدم وجود تفاعل كبير بين اثنين من العوامل، تم استخدام بيانات متوسط في شرطين التلقيح وبدون التلقيح، لرسم الرسوم البيانية. لمحتوى النيتروجين في الحبوب، لأن التفاعل بين اثنين من العوامل كان كبيرا، ووضعت رسوم بيانية منفصلة. كان تأثير تلقيح البذور بالبكتريا معنويا على محتوى النيتروجين في الحبوب وطول الأذن. حيث كان الفوسفور بمعدل 187/5 كيلوغرام في الهكتار المقدار الأمثل في الزيادة محتويه في محصول الحبوب، وزن الألف حبة، الغلة البيولوجية، مساحة الدوراق، ومحتوى النيتروجين وامتصاص الفسفور بوساطة الحبوب وطول العرنوس. أشارت النتائج أيضا إلى أن تلقيح البذور بالازونوباكتر الى زيادة محتوى النيتروجين في الحبوب، امتصاص النيتروجين بوساطة الحبوب و طول العرنوس. لذا، فإن استخدام التثبيت الحيوي للنايتروجين مع سماد الفسفور الكيمياوي ممكن أن يكون استراتيجية للوصول إلى الزراعة المستدامة.

الكلمات الدالة: الذرة ، تثبيت النيتروجين ، الآزوتوباكتر، امتصاص، المكون العائد.

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