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Effects of phosphate solubilizing bacteria and phosphorous levels on rice (*oryza sativa l.*)

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A B S T R A C T

In order to study the effects of phosphate solubilizing bacteria and of rates of phosphorous application on the growth and yield of rice cultivar Shiroodi, an experiment was conducted in the split plot format using the randomized complete block design in four replications in the city of Neka of northern Iran in 2012. The experimental treatments included three rates of phosphorous application (zero, 83, and 165 Kg.h) in the form of concentrated superphosphate as the major factor, and four types of biofertilizers (not inoculated with bacteria, inoculated with Pseudomonas fluorescens, inoculated with Pseudomonas putida, and simultaneously inoculated with both Pseudomonas fluorescens and Pseudomonas putida) as the minor factor. Results obtained indicated that application of mineral phosphorous caused an increase in seed yield of the rice cultivar Shiroodi so that the maximum seed yield (630.251 g.m2) was obtained in the treatment of applying 165 Kg.h phosphorous. The increase of seed yield in this treatment was accompanied with the maximum number of tillers (23.981), the longest panicles (27.209 cm), and the largest number of seeds per panicle (116.30). It was also observed that inoculation with bacteria improved the yield and yield components of the rice cultivar Shiroodi so that the maximum number tillers (22.46), the highest percentage of fertile tillers (94.64), the longest panicles (26.29 cm), and the largest number of seeds per panicle (104.95) were observed when seeds were simultaneously inoculated with both bacterial species. With respect to seed yield, it was found that the use of a single bacterial species, as compared with the employment of two bacterial strains, caused a greater increase in seed yield so that the maximum seed yields of 594.97 and 594.85 g.m2 were obtained when the putida or the fluorescens species were used, respectively. With regard to the Interaction effects of the application of mineral phosphorous and inoculation with bacteria also, the highest seed yield (696.91 g.m2) was observed under the interaction effects of applying 83 Kg of mineral phosphorous and inoculating with Pseudomonas putida. This was accompanied by the maximum percentage of fertile tillers (94.24%), long panicles (26.09 cm), and a considerable 1000 –seed weight (22.80 g).

1. Introduction

Phosphorous is an essential element absorbed from the soil in the form of phosphate. Combination of phosphorous with substances present in soils limits the distribution of phosphorous to the root system; therefore, phosphorous may not be available to plants even when its concentration in soils is high (Vance et al., 2003; Hammond et al., 2004; Raghothama et al., 2005). Hence, widespread use of chemical fertilizers and other chemicals is one of the main environmental problems; and it also increases the cost of producing crops (Ghasemi et al., 2012). Moreover, applying excessive rates of chemical phosphorous fertilizers adds to soil erosion and, hence, leads to the generation of runoff (Park et al., 2009). Phosphate solubilizing bacteria increase the available phosphates in rice fields; and this acts as a plant growth promoting mechanism (Verma et al., 2001). Plant growth promoting rhizosphere bacteria can be a suitable replacement for this purpose. These are a group of rhizosphere bacteria that increase plant growth and yield through their cloning activities on plant roots (Gholami et al., 2009). The plant growth mechanisms mediated by these bacteria have not been completely elucidated, but it seems that their capability in producing phytohormones (Egamberdiyeva, 2007; Shaharoona et al., 2006), in biologically fixing nitrogen (Salantur et al., 2006), in making sidrophores, in synthesizing antibiotics, enzymes or fungicides, and also in solubilizing mineral phosphorous and other plant nutrients, improves plant growth (Gholami et al., 2009). The benefits of inoculating plants with bacteria include increases in several indices such as rate of germination, root growth, yield per unit area, bio-control of disease causing agents, leaf area, chlorophyll content, resistance to drought, weight of aerial parts and roots, and microbial activities (Lucy et al., 2004). Bacteria belonging to the genus Pseudomonas, particularly Pseudomonas fluorescens and Pseudomonas putida, are among the most important members of rhizosphere bacterial community that substantially increase the quantity of phosphorous absorbed by crop plants (Abbaszadeh , 2009). These bacteria increase the growth of rice plants. In rice inoculated with these bacteria, the root dry weight, the height of plants, the dry weight of aerial parts, and the height at which seeds are produced, significantly increase compared to the control (which has not been inoculated) at the one percent probability level (Ashrafuzzaman et al., 2009). Rahmati Khorshidi et al. (2011) showed that the 1000 – seed weight, and also the seed yield, increased significantly when rice was inoculated with Pseudomonas fluorescens. Researchers studied the morphological and physiological features of the rice cultivar Tarom and stated that inoculation of rice seeds with Pseudomonas bacteria resulted in an increase in the development and growth of rice plants and in the allocation of more photosynthates to seeds, causing a rise in the number of seeds per panicle, in the number of panicles per plant, and in the 1000 – seed weight and, hence, in seed yield (Abbaszadeh, 2009). Mirzaee Heidari et al. (2009) found that strains of putida and fluorescens species of Pseudomonas bacteria induced an increase in the length of roots, in the germination rate, and in seed yield of wheat. In the research conducted by Gholami and Nezarat (2011), it was shown that the dry weight of the whole corn plants and the dry weight of the seeds significantly rose by inoculation with Pseudomonas bacteria. Kausar and Shahzad (2006) demonstrated that inoculation of corn with Pseudomonas bacteria (P. fluorescens and P.

putida) under saline conditions significantly increased the weight of the aerial parts as compared with the control. Cong et al. (2011) conducted a study concerning the effects of biofertilizers on rice and discovered that application of biofertilizers alone brought about an increase in rice seed yield. Therefore, we conducted this experiment in the cropping year 2011 – 2012 in the city of Neka to study and compare the effects of the strains of Pseudomonas fluorescens and Pseudomonas putida (used singly and in combination) with those of applying different rates of mineral phosphorous, and with those of combined inoculation with these bacteria and application of phosphorous.

2. Materials and methods

This experiment was conducted in the region of Chaman of the city of Neka in the summer of 2012 in the format of split plots using the randomized complete block design in four replications (a total of 48 plots). The factors studied included three rates of mineral phosphorous in the form of concentrated superphosphate (zero, 83, and 165 Kg.h phosphorous) as the main factor, and four types of inoculation of the seedlings of the rice cultivar Shiroodi (the control that was not inoculated, inoculation with Pseudomonas fluorescens, inoculation with Pseudomonas putida, and simultaneous inoculation with these two species of bacteria) as the minor factor. Seedlings were transplanted in six rows twenty cm apart in six square meter plots in which each transplant contained four seedlings. A composite sample of soil from the surface of the ground down to the depth of 30 cm was taken and sent to a laboratory for determining the physical and chemical features of the soil and for measuring the content of plant nutrients (especially that of phosphorous). Rice seedlings were inoculated with the selected bacteria according to the type of the treatment. Rates of application of phosphorous were also determined according to the type of the treatment; and phosphorous was applied before planting the seedlings. All operations during the growth of the plants were carried out as is customary in the region. Urea was applied at three stages at a total rate of 200 Kg.h; and potassium was incorporated into the soil at the rate of 100 Kg.h at the end of tillering and the start of flowering. Harvest was carried out at full physiological maturity and yield was measured at 14 percent moisture content. At crop maturity, ten plants were randomly selected from each plot and their seed yield and yield components (including the number of tillers per plant, the number of panicles per plant, the length of the panicles, the number of seeds per panicle, and the 1000 – seed weight) were measured. Analysis of the variance was performed using the software MSTATC, and comparison of the means of the data was performed on the basis of Duncan's multiple range tests at the five percent probability level.

3. Results and discussion

In cereals, tillering is influenced by hormonal factors; and the exogenous cytokinin (produced by PGPR bacteria) causes an increase in the number of tillers in wheat because this material is necessary for cell division in tiller buds (Johnston and Jeffcoat, 1977). Therefore, PGPR bacteria, as producers of the hormone cytokinin, can be very effective in increasing the number of tillers (Abbaszadeh, 2009). In our study, results obtained from analysis of the variance of the data indicate that inoculation of rice seedlings with growth promoting bacteria causes significant increases in the number of tillers. This finding is in conformity with results obtained in the experiment conducted by Abbaszadeh (2009). Results we observed from analysis of the variance of the data showed that the main effects of mineral phosphorous and those of phosphorous solubilizers were significant at the one percent level of probability, but that there were no significant changes in the Interaction effects of the two factors (Table 1). Comparison of the means of the data suggested that the number of rice tillers increased when higher rates of phosphorous fertilizers were applied so that the maximum number of tillers (23.98) was obtained with the application of 165 Kg.h of phosphorous (Table 2). The main effects of the phosphorous solubilizing bacteria also increased the number of tillers per plant. The largest numbers of tillers (22.467 and 21.075) were obtained with the simultaneous inoculation of the seedlings with the two bacterial species and with the inoculation of the seedlings with Pseudomonas putida, respectively (Table 3). These results also indicated that, under the interaction effects of applying 165 Kg.h of phosphorous and of inoculation with both bacterial species, the maximum number of tillers (26.375) was observed, although this increase was not statistically significant (Figure 1).

The table of analysis of the variance suggests that the effects of the rates of mineral phosphorous application and the use of phosphate solubilizers, and also their Interaction effects, on the feature of fertile tillers were significant at the one percent probability level (Table 1). Comparison of the means of the main effects of phosphorous suggested that the percentage of fertile tillers significantly increased when higher rates of phosphorous were applied, that this percentage reached its maximum of 93.75 percent when 83 Kg of phosphorous were used per hectare, and that the percentage of fertile tillers declined when higher rates of phosphorous were applied so that it decreased to 92.80 percent at 165 Kg.h phosphorous (Table 2). Results obtained also showed that the percentage of fertile tillers increased under the main effects of bacterial inoculation and reached its maximum of 94.64 percent when the seedlings were inoculated with both bacterial species (Table 3). It was also observed that the minimum percentage of fertile tillers (92.01 %) was obtained under the Interaction effects of 165 Kg.h of phosphorous and the simultaneous inoculation with both bacterial species, and the maximums (94.24 and 94.18 %) when 83 Kg.h of phosphorous were applied and the seedlings were inoculated with Pseudomonas putida, or with Pseudomonas fluorescens, respectively (Figure 2).

One of the main effects of phosphorous on plants is on their reproductive factors, particularly on their sexual and reproductive organs. In our experiment also, it was observed that the length of the panicles significantly increased under the influence of mineral phosphorous (Table 1) so that it reached 26.044 cm when 83 Kg of phosphorous were applied (it increased to 27.209 cm with the application of 165 Kg.h of phosphorous; however, this increase was not statistically significant) (Table 2). The main effects of the bacteria on the length of panicles were significant compared to the control, but the differences between the effects of the two species, or between the effects of either of the two species and the simultaneous application of both species were not significant. The longest panicles (26.29 cm) were obtained in the treatment of simultaneous inoculation with both bacterial species (Table 3). Comparison of the means of the Interaction effects of the two factors indicates that the shortest panicles (18. 843 cm) belonged to the control (no phosphorous application and no inoculation), and the longest to the treatment of applying 165 Kg.h of phosphorous and simultaneous application with both bacterial species (27.83 cm), or to the treatment of inoculation with Pseudomonas putida (27.77 cm) (Figure 3).

Phosphorous is one of the essential macronutrients and its deficiency severely restricts plant growth. Phosphorous is also very important in flowering and in seed set. The main effects of mineral phosphorous and phosphate solubilizers on the number of seeds per panicle were significant at the one percent probability level, but the Interaction effects of phosphorous application and inoculation on the number of seeds per panicle were not significant (Table 1). These results are in agreement with those in the report submitted by Ghasemi et al. (2012). This shows that phosphorous greatly affects the reproductive factors; and Pseudomonas bacteria have demonstrated their ability in increasing the phosphorous content of plants (and, thereby, in providing sufficient phosphorous for flowering and for seed set). The table of comparison of the main effects of phosphorous suggests that raising the rate of phosphorous application to 83 and 165 Kg.h significantly increased the number of seeds, to 96.05 and 116.3 per panicle, respectively (Table 2). In the comparison of the main effects of phosphate solubilizing bacteria, it was observed that the largest and the smallest numbers of seeds per panicle were those of the control and of the treatment of simultaneous inoculation of the seedlings with both bacterial species, respectively (Table 3). Figure 4 shows that the Interaction affects of the two factors in this study caused an increase in the number of seeds per panicle; however, this increase was not significant. The minimum number of seeds per panicle (50.25) belonged to the control plots, and the maximum (126.95) to the treatment of the rate of application of 165 Kg.h of phosphorous and simultaneous inoculation with both bacterial species.

Seed weight is another component of yield, and it is more influenced by the related genotype. Of course, environmental conditions, particularly at the stage following seed set, considerably influence the 1000 – seed weight (Abbaszadeh, 2009). Table 2 indicates that the main effects of the fertilizer treatment did not bring about a rise in the 1000 – seed weight, but the 1000 – seed weight increased when 83 Kg of mineral phosphorous were applied; however, when the rate of phosphorous application was raised to 165 Kg.h, the 1000 – seed weight decreased to 22.59 g and declined to its minimum in the fertilizer treatment (which could be attributed to the increase in the number of seeds per panicle and to the effect of partitioning). The table of analysis of the variance shows that the Interaction effects of the two factors, and the main effects of the treatment of inoculation with bacteria, were significant at the one percent probability level. Biswas et al. (2000) reported that inoculating rice (Oryza sativa L.) seedlings with growth promoting rhizosphere bacteria significantly increased phosphorous absorption (by 10 to 28 %) compared to the control. Therefore, Pseudomonas bacteria improve photosynthesis by increasing phosphorous absorption and transport to plant cells, and cause larger seeds to be produced through transporting sufficient photosynthates to the seeds at the seed – filling stage. This leads to a rise in the 1000 – seed weight and, hence, to an increase in seed yield. In Table 3, the minimum 1000 – seed weight (22, 36 g) belonged to the control, and the maximum (23.01 g) to the treatment of inoculation with Pseudomonas fluorescens. Statistical comparison of the Interaction effects of the two factors suggests that the largest 1000 – seed weights (23.50 and

23.28 g) were observed in the treatment of no phosphorous application and simultaneous inoculation with both bacterial species, and the treatment of inoculation with Pseudomonas fluorescens and application of 83 Kg of mineral phosphorous per hectare, respectively, while the minimum 1000 – seed weights (22.25 and 22.27 g) belonged to the control treatment and to the treatment of applying 165 Kg of phosphorous without any inoculation, respectively (Figure 5).

Table 1

Analysis of variance of features of phosphorous solubilizing bacteria and mineral phosphorous studied.

Sources of change	Degree of freedom	Seed yield	Number of tillers	Percentage Of fertile tillers	Length Of the panicles	Number Of seeds per panicle	1000– seed weight
Replication	3	1356.392	2.769	0.078	2.189	4.437	0.614
Mineral phosphorous	2	140179.751**	185.981**	3.652**	93.198**	9239.902**	0.184
Error	6	1178.65	4.321	0.045	1.975	13.539	0.248
Phosphate solubilizers	3	10871.770**	26.531**	2.009**	18.718**	1721.907**	1.009**
Interaction effects	6	4683.233**	1.352	0.527**	5.332**	20.995	0.519**
Error	27	718.550	2.912	0.103	0.572	22.346	0.134

** refer to significance at the five and one percent probability levels, respectively.

Table 2

The effects of the treatment of applying mineral phosphorous on yield and on features related to yield.

Main effects	Seed yield	Number of tillers	Percentage of fertile tillers	Length of the panicles	Number of seeds per panicle	1000 – seed weight
			Recommended mineral phosphorous			
Zero	457.877b	17.181c	93.181b	22.570b	68.431c	22.744a
83 kilograms	625.192a	21.019b	93.756a	26.044a	96.056b	22.806a
165 kilograms	630.251a	23.981a	92.806c	27.209a	116.304a	22.597a

Figures in each group under each column having at least one letter in common are not significantly different on the basis of Duncan's multiple range test at the five percent probability level.

Table 3

The effects of the treatment of phosphate solubilizing bacteria on yield and features related to yield.

Main effects	Seed yield	Number of tillers	Percentage of fertile tillers	Length of the panicles	Number of seeds per panicle	1000 –seed weight
Treatment of inoculating seedlings						
Not inoculated	532.280c	18.875c	93.437a	23.488c	77.214d	22.362c
Pseudomonas putida	594.972a	21.075ab	93.371a	25.941ab	99.317b	22.608bc
Pseudomonas fluorescens	594.851a	20.492b	93.547a	25.379b	92.908c	23.016a
Inoculation with both bacterial species	562.325b	22.467a	94.646b	26.290a	104.950a	22.877ab

Figures in each group under each column having at least one letter in common are not significantly different on the basis of Duncan's multiple range test at the five percent probability level.

Table 4
Interaction effects of mineral phosphorous and phosphate solubilizing bacteria on the rice cultivar Shiroodi.

Main effects	Seed Yield (g.m2)	Number of tillers	Percentage of fertile tillers	Length of the panicles	No. of seeds per panicle	1000-seed weight (g)
Mineral Phosphorous and Bacteria						
P0 x B0	435.502f	15.700a	93.433bc	18.843e	50.250a	22.250d
P0 x P. putida	470.888f	17.550a	93.234bc	23.957d	76.225a	22.338cd
P0 x P. fluorescens	455.897f	17.250a	93.129bcd	23.165d	65.650a	22.887bc
P0 x P. putida and P. fluorescens	469.218f	18.225a	92.957cd	24.315d	81.600a	23.500a
P1 x B0	560.582e	19.450a	93.630b	25.580c	82.025a	22.562cd
P1 x P. putida	696.912a	21.550a	94.242a	26.095bc	100.950a	22.800bcd
P1 x P. fluorescens	648.054bc	20.275a	94.189a	25.780c	94.950a	23.287ab
P1 x P. putida and P. fluorescens	595.219de	22.800a	92.963cd	26.720abc	106.300a	22.575cd
P2 x B0	600.753de	21.475a	93.247bc	26.040bc	99.367a	22.275cd
P2 x P. putida	617.115cd	24.125a	92.637d	27.770a	120.775a	22.687cd
P2 x P. fluorescens	680.601ab	23.950a	93.323bc	27.193ab	118.125a	22.872bc
P2 x P. putida and P. fluorescens	622.573cd	26.375a	92.019e	27.835a	126.950a	22.555cd

Figures in each group under each column having at least one letter in common are not significantly different on the basis of Duncan's multiple range test at the five percent probability level.

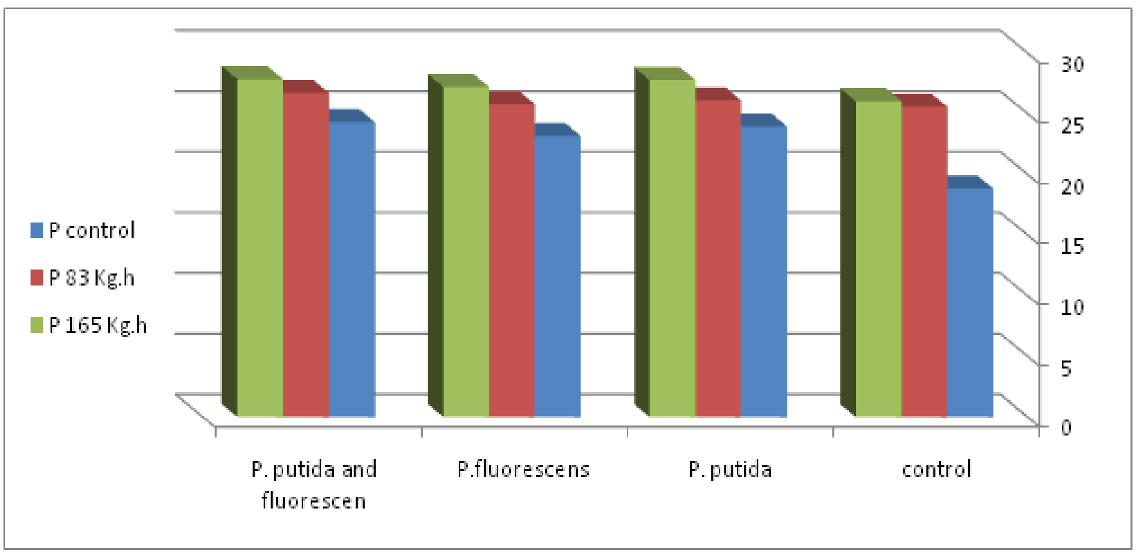

Fig. 1. Interaction effects of mineral phosphorous and phosphate solubilizing bacteria on the number of tillers per plant.

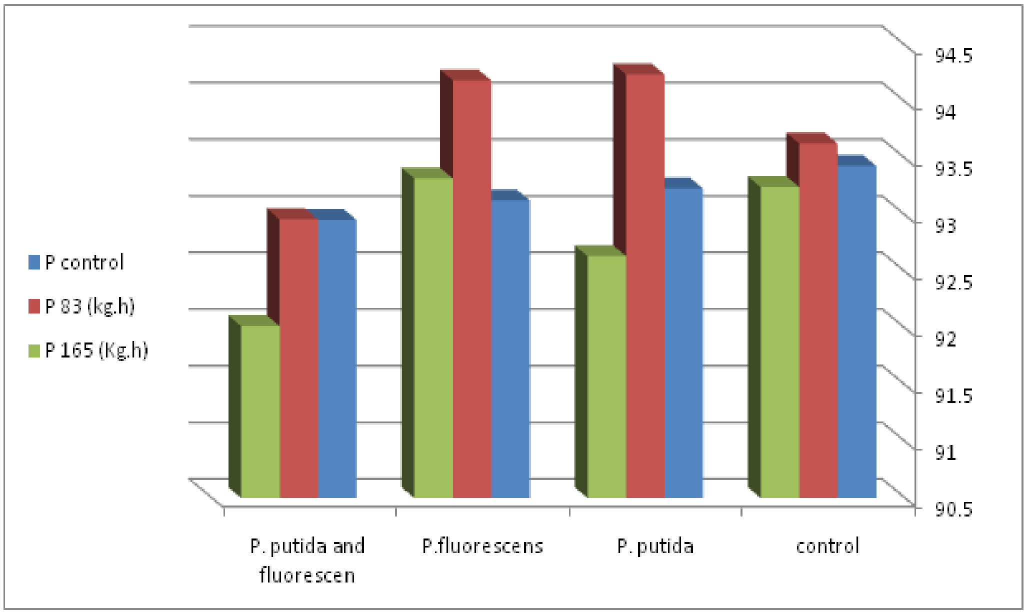

Fig. 2. Interaction effects of mineral phosphorous and phosphate solubilizing bacteria on the percentage of fertile tillers.

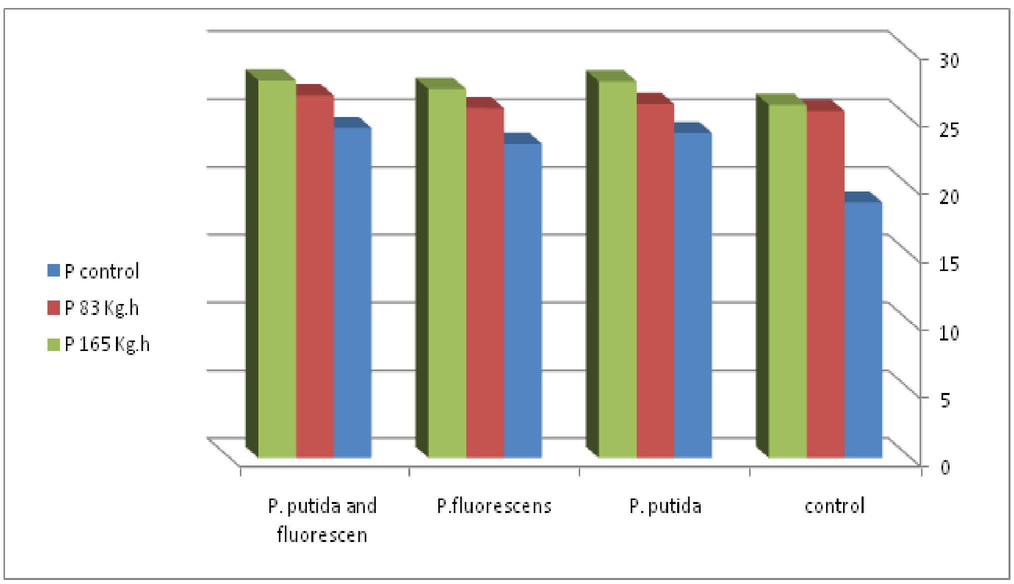

Fig. 3. Interaction effects of mineral phosphate and phosphate solubilizing bacteria on the length of panicles.

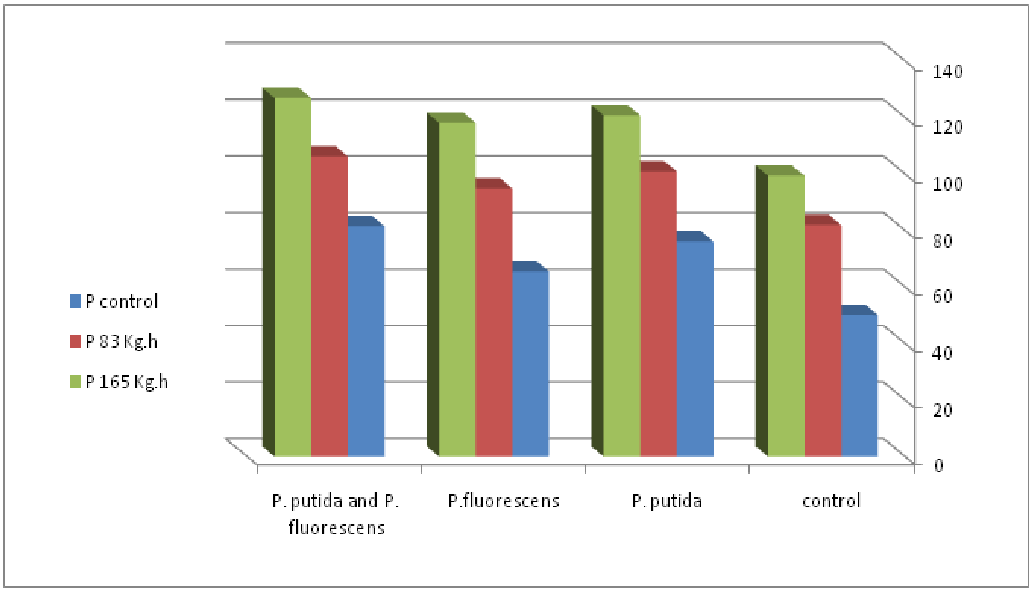

Fig. 4. Interaction effects of mineral phosphorous and phosphate solubilizing bacteria on the number of seeds per panicle.

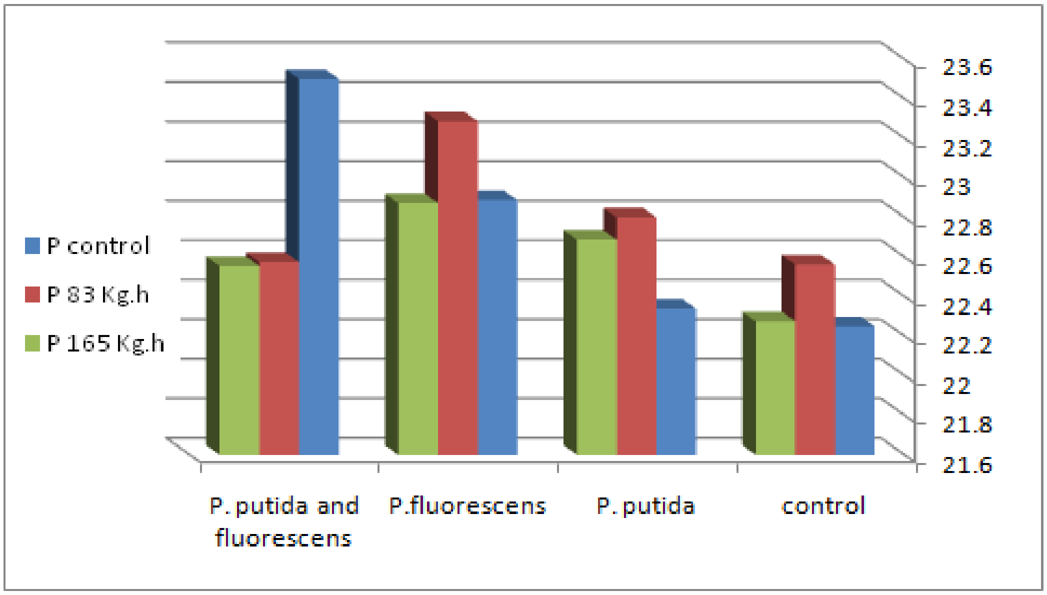

Fig. 5. Interaction effects of mineral phosphorous and phosphate solubilizing bacteria on the 1000-seed weight.

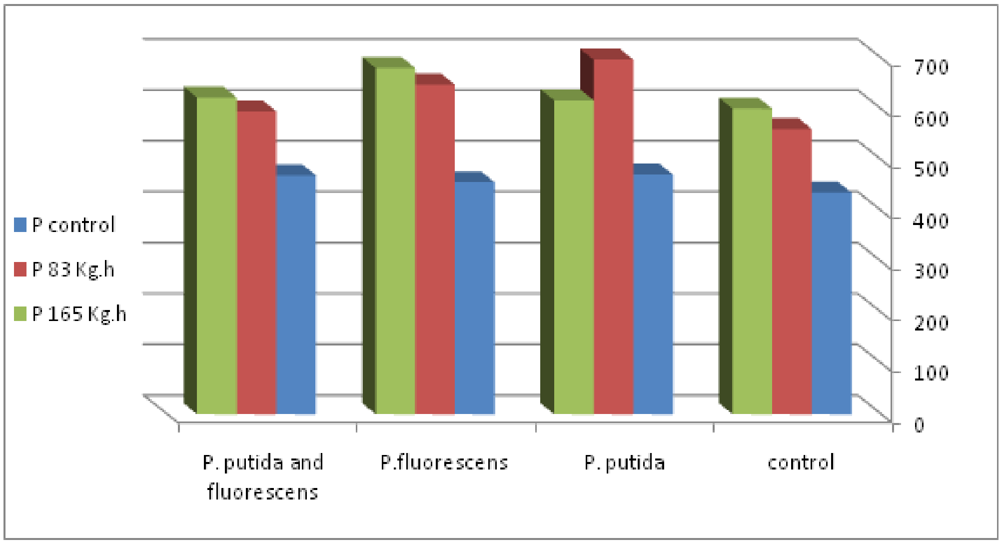

Fig. 6. Interaction effects of mineral phosphorous and phosphate solubilizing bacteria on seed yield (g.m2).

In our research, inoculation of rice seedlings with Pseudomonas bacteria led to an increase in the development and growth of rice plants and to allocation of a larger amount of photosynthates to the seeds (which resulted in an improvement in yield components such as longer panicles, larger number of seeds per panicle, greater 1000 – seed weight and, hence, a higher seed yield). Analysis of the variance of the data indicated that the main effects of mineral phosphorous and those of Pseudomonas bacteria, and also their Interaction effects, significantly increased yield at the one percent probability level. These results agree with those obtained by Rahmati Khorshidi et al. (2011). In our study, raising the rate of phosphorous fertilizer application increased yield, but the differences in yield resulting from the application of 83 or 165 Kg.h of phosphorous were not significant (i. e. not all of the phosphorous applied to the soil is absorbed by plants). On the contrary, comparison of the means of the effects of inoculation revealed that the maximum yield (562.32 g.m2) was achieved when the seedlings were simultaneously inoculated with both bacterial species (Table 3). However, the Interaction effects of the two factors showed that the largest yield (606.91 g.m2) was obtained in the treatment of applying 83 Kg.h of the phosphate fertilizer and inoculation with Pseudomonas putida, but that yield declined (and reached 617.11 g.m2) in the treatment of applying 165 Kg.h of the phosphate fertilizer. This indicates that inoculation with Pseudomonas putida increases phosphorous absorption by plants, and that raising the rate of phosphorous application leads to plant poisoning (and to a reduction in yield according to Micherlich's law of diminishing returns) (Figure 6). Moreover, the minimum yield (435.50 g.m2) belonged to the control treatment. Inoculation with bacterial species in plots with no phosphorous application caused a rise in yield, with the maximum yield obtained in the treatment of inoculation with Pseudomonas putida. However, this increase was not statistically significant (Table 4).

4. Conclusion

In our study, it was observed that application of phosphorous increased the seed yield of the rice cultivar Shiroodi so that the highest seed yield (630.251 g.m2) was accompanied by the largest number of tillers (23.98), the longest panicles (27.20 cm), and the maximum number of seeds per panicle (116.30). We also found that inoculation with Pseudomonas bacteria (P. fluorescens and P. putida) improved yield and yield components so that the maximum number of tillers (22.46), the highest percentage of fertile tillers (94.64), the longest panicles (26.29 cm), and the largest number of seeds per panicle (104.95) were achieved by inoculating rice seedlings with both bacterial species. With regard to seed yield, it was observed that inoculation with one of the two bacterial species, as compared with inoculating seedlings with both bacterial species, had greater effects in increasing seed yield so that the maximum seed yields (594.97 and 594.85 g.m2) were obtained by inoculating rice seedlings with Pseudomonas putida or Pseudomonas fluorescens, respectively. The Interaction effects of applying phosphorous and inoculating with bacteria resulted in the maximum seed yield (696.91 g.m2) when mineral phosphorous was applied at 83 Kg.h and seedlings were inoculated with Pseudomonas putida. This was accompanied by the highest percentage of fertile tillers (94.24), long panicles (26.09 cm), and a large 1000 – seed weight (22. 80 g).

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