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Effects of Phosphate Solubilizing Bacteria and Mineral Phosphates on Hyola 401 Yield and Yield Components

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ABSTRACT: Comparison of the means showed that the effects of bacterial inoculation on number of seeds per pod, number of pods on the main stem, and seed yield were significant at the 1% probability level. Moreover, rates of phosphate application had significant effects on all traits but plant height and height of harvest by combine at the one percent level. Comparing the means of the effects of inoculation on seed yield indicated the maximum seed yield (2610 kg/ha) belonged to the treatment of inoculation with *Pseudomonas putida*, which was not significantly different from that of simultaneous seed inoculation with both *putida* and *fluorescens* strains (2473 kg/ha). The minimum seed yield was that of the treatment in which seeds were not inoculated (2224 kg/ha). Comparing the means related to the effects of phosphate application rates on seed yield indicated the largest seed yield (2840 kg/ha) was achieved at 75 kg/ha, and the smallest (1810 kg/ha) when phosphate was not applied. Comparison of the interaction effects of inoculation and phosphate application revealed that the highest seed yield (2980 kg/ha) was obtained in the treatment of simultaneous inoculation with both *P. putida* and *P. fluorescens* and phosphate application rate of 75 kg/ha. This highest seed yield was not significantly different from that of the treatment in which phosphate was applied at 75 kg/ha. The lowest seed yield (1480 kg/ha) was observed in the treatment of inoculation with *P. putida* without phosphate application, which was not significantly different from that of the treatment of no inoculation and no phosphate application (1600 kg/ha).

***Keywords*:** Canola, *Pseudomonas putida*, *Pseudomonas fluorescens,* Phosphorous

INTRODUCTION

Phosphorous is the second most important plant macronutrient after nitrogen, it is present in soils in organic and mineral forms, and is absorbed from soils as phosphates (Subbarao, 1988). However, phosphorous combines with other soil materials and these combined forms limit phosphorous movement in soils. Therefore, phosphorous becomes unavailable to plant root system, even when soil phosphorous concentration is high. To cope with this situation, plants use various methods to free soil phosphorous so that they can absorb it (Raghothama, 2005; Hammond et al., 2004; Vance et al., 2003). When plants face phosphorous deficiency, they increase carbohydrate entry into roots, which increases the root/shoot ratio (Sezai et al., 2006).

Advantages of inoculating plants with growth stimulating bacteria include enhancing numerous indicators such as germination rate, root growth, yield per unit area, biological control of pathogens, leaf area, chlorophyll content, drought resistance, weight of aerial parts and roots, and microbial activities (Lucy et al., 2004).

Inoculating corn and sorghum with *Azospirillum* bacteria showed these bacteria increased phosphorous absorption in these plants through expanding their root systems (Fallik and Okon, 1988).

Corn seed inoculation with *Azospirillum* increased phosphate ion absorption by 50 to 70% compared to the control. It was concluded that this increase in ion absorption (resulting from inoculation with *Azospirillum*) could play an important role in increasing plant growth and that, under conditions of nutrient deficiency, *Azospirillum* could increase the efficiency of nutrient absorption through helping plants to absorb nutrients. Wheat phosphorous content increased considerably in the presence of *Azotobacter* and *Pseudomonas* striata (Almas and Saghir, 2005).

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Utilization of rhizosphere microorganisms to increase yield and quality of agricultural products, and to control plant diseases, has interested researchers (Hammond et al., 2004). The rapid effect of applying chemical fertilizers on increasing plant growth reduced research activities conducted in the science of soil microbiology but, with the gradual recognition of the adverse and destructive effects resulting from uncontrolled application of these fertilizers on the environment, greater attempts were made to apply this science. Nowadays, the ability of soil-borne microorganisms in releasing plant nutrients such as phosphorous and potassium, in decomposing complex organic compounds, and in controlling pests and diseases of plants, is well utilized in countries that are pioneers in sustainable agriculture. Pseudomonas bacteria, especially P. putida and P. fluorescens, are among the most important members of rhizosphere bacteria, and considerably increase absorption of phosphorous by plants. This research studied the effects of phosphate solubilizing bacteria on absorption of insoluble phosphorous in soil, compared the effects of these bacteria with those of mineral phosphate application, and investigated the possibility of replacing mineral phosphate by seed inoculation with these bacteria in colza cultivar Hyola 401 planted at the optimal planting date in Dasht-e Naz in Sari.

MATERIALS AND METHODS

An experiment was conducted at the Dasht-e-Naz Agronomy Research Station (Agriculture and Natural Resources Research Center of Mazandaran) in the cropping year 2012-2013. This station is 10 km from Miandorood and 35 km from Sari, the capital city of Mazandaran province. The experimental site has an altitude of 13.5 m, longitude of 53 ˚11″ and latitude of 36 ˚04″ north. The average annual rainfall at the station is 685 mm, and it has a temperate Caspian climate and (according to the statistics of the Meteorological Organization of Mazandaran) maximum and minimum annual temperatures of 27.3 and 7.1 C˚.

The experiment was in the factorial arrangement using the randomized complete block design with three replications. Four levels of bacteria (the control, *Pseudomonas putida, Pseudomonas fluorescens*, and simultaneous use of both bacterial species) were the treatments in the main plots, and five levels of mineral phosphorous application (the control, 25, 50, 75, and 100 kg/ha of phosphorous in the form of concentrated superphosphate) the treatments in the sub plots. Each experimental plot included five 5-m lines 30 cm apart. The distance between adjacent plots was 1 meter and between replications 2 meters (used as passage way).

RESULTS AND DISCUSSION

Plant height: The results related to plant height showed the individual effects of inoculation levels and phosphate application rates, and their interaction effects, on plant height were not significant (Table 1). Comparison of the means related to inoculation indicated the tallest plants (106 cm) belonged to the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens*, and the shortest (101.4 cm) to the control (no bacterial inoculation) (Table 2). As for phosphate application, the tallest plants (106.2 cm) were observed in the treatment of applying phosphate at 25 kg/ha, and the shortest (100.8 cm) in the control (no phosphate application) (Table 2). Comparing the means of the interaction effects of inoculation with phosphate solubilizing bacteria and phosphate application on plant height showed that the shortest plants (95.3 cm) belonged to the treatment of inoculation with *P. fluorescens* and phosphate application rate of 50 kg/ha.

Table 1. ANOVA of the data related to the studied traits

Sources of Variation	Degree of Freedom	Mean Squares							
		Plant height	Height harvest by combine	Number of lateral shoots	Number of pods on the main stem	Number of pods per plant	Length of pods	Number of seeds per pod	Seed yield
Replication	2	38.834	21.155	0.002	75.266	951.122	1.604	92.266**	1481.867 ns
Rate of phosphate	3	61.324	9.131	0.487	216.886*	537.878	0.155	45.439*	54840.283**
Error	6	102.748	27.499	0.302	34.560	391.629	0.411	8.757	1572.133
Level of bacterial inoculation	4	72.389	5.867	1.161**	138.526**	2528.918**	0.792**	74.423**	96978.942**
Interaction effects	12	77.678	4.304	0.063	0,063	123.512	0.691	4.962	1748.908
Error	32	46.809	5.594	0.052	0.052	152.103	1.630	5.072	9065.379
Coefficient of variation (%)	-	6.61	5.71	5.64	5.64	6.87	5.01	7.58	10.16

Table 2. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on plant height (cm)

Inoculation level/ Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	95.733	98.000	107.000	102.333
25	107.86	107.733	102.667	106.600
50	101.533	101.533	105.933	97.000
75	95.933	108.200	110.000	109.67
100	106.067	105.933	97.000	103.267

This was not significantly different from that of the control (95.7 cm) with no inoculation or phosphate application. The tallest plants (110 cm) were observed in the treatment of inoculation with *P. fluorescens* and phosphate application rate of 75 kg/ha (Table 2).

Height of harvest by combine: The results indicated the individual effects of inoculation with phosphate solubilizing bacteria and phosphate application or their interaction effects on height of harvest by combine were not significant (Table 1). Comparison of the means related to inoculation revealed that the maximum height of harvest by combine was that of the control (no inoculation) at 42.4 cm, and the minimum (40.7 cm) that of the treatment in which seeds were inoculated with *P. fluorescens* (Table 3). Rates of phosphate application did not significantly influence height of harvest by combine either. The maximum and minimum heights of harvest by combine (42.30 cm, 40.7 cm) were observed in the treatments of applying phosphate at 25 and 75 kg/ha, respectively. The interaction effects of inoculation and phosphate application on height of harvest by combine were greatest (with height of harvest by combine of 43.2 cm) in the treatment of no inoculation and application of phosphate at 50 kg/ha. These effects were smallest (with height of harvest by combine of 38.7 cm) in the treatment of inoculation with *P. putida* and application of phosphate at 50 kg/ha (Table 3).

Table 3. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on Height of harvest by combine (cm)

Inoculation level / Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	40.73	42.53	41.66	43.06
25	42.73	42.93	41.46	42.06
50	43.20	38.73	39.80	41.86
75	42.60	39.60	40.46	40.13
100	43	40.73	40.26	40.73

Number of lateral shoots: The results of analysis of variation showed that only the effects of phosphate application rate were significant at the 1% level, but those of inoculation and of the interaction effects of phosphate application and inoculation were not (Table 1). The maximum number of lateral shoots in inoculation treatments (4.23) belonged to the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens* (which was not statistically different from those of the other treatments), and the minimum (3.8) to the control (no inoculation) (Table 4).

Table 4. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on number of lateral shoots

Inoculation level / Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	3.4	3.46	3.46	3.69
25	3.8	4.20	4.40	4.46
50	3.86	4.06	4.46	4.33
75	4.23	4.20	4.33	4.20
100	3.86	4.06	4.20	4.46

As for the effects of phosphate application, the largest number (4.24) was that of applying phosphate at 75 kg/ha (which was not statistically different from those of the treatments in which phosphate was applied at 25, 50, or 100 kg/ha). The minimum number of lateral shoots (3.5) was that of the control (no phosphate application) (Table 4). Comparing the means of the interaction effects of phosphate application and inoculation revealed that the smallest number of lateral shoots (3.4) was obtained in the control (no phosphate application and no inoculation), and the largest (4.46) in the treatment of inoculation with P. fluorescens and application of phosphate at 50 kg/ha (Table 4).

Number of pods on the main stem: The results indicated that the effects of phosphate solubilizing bacteria on number of pods on the main stem were significant at the 5% probability level (Table 1), and that the effects of applying phosphate on this trait were significant at the 1% probability level. However, the interaction effects of phosphate application and inoculation on this trait were not significant (Table 1). Comparison of the means related to inoculation revealed that the maximum number of pods on the main stem (84.6) belonged to the treatment of inoculation with *P. fluorescens* (which was not statistically different from 83.5 observed in the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens*). The minimum number of pods on the main stem (77.45) was that of the treatment in which seeds were inoculated with *P. putida*, which was not statistically different from 77.65 observed in the treatment with no inoculation (Table 4). Comparison of the means related to phosphate application indicated the lowest number of pods on the main stem (74.8) was observed in the treatment of no phosphate application. The highest number of pods on the main stem (82.98) belonged to the treatment of applying phosphate at 100 kg/ha, which was not statistically different from those obtained with the application of phosphate at 25, 50, or 75 kg/ha. Comparing the means of the interaction effects of phosphate application and inoculation showed the smallest number of pods on the main stem (71.8) was that of the control (no phosphate application and no inoculation), and the largest (88.6) that of the treatment in which seeds were inoculated with P. fluorescens and phosphate was applied at 100 kg/ha (Table 5).

Table 5. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on number of pods on the main stem

Inoculation level / Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	71.86	74.80	78.67	74.80
25	79.20	77.53	83.86	87.53
50	74.66	77.26	87.13	85.86
75	82.80	77.33	85.66	86.06
100	79.73	80.33	86.60	83.26

Number of pods per plant: The results showed that only the effects of phosphate application rate on the number of pods per plant were significant at the 1% probability level, and that those of phosphate solubilizing bacteria or of the interaction effects of bacterial inoculation and phosphate application were not (Table 1). Comparison of the means related to inoculation indicated that the maximum number of pods per plant (187.5) belonged to the treatment of inoculation with *P. fluorescens*, and the minimum (174.7) to the treatment of no inoculation (Table 6). Comparison of the means related to phosphate application revealed that the largest number of pods per plant (191.2) was that of the treatment in which phosphate was applied at 75 kg/ha. This largest number was not statistically different from 189.8 that was obtained at application rate of 50 kg/ha. The smallest number of pods per plant (155.2) was observed when no phosphate was applied (Table 6). Comparison of the means related to the interaction effects of inoculation and phosphate application indicated the lowest number of pods per plant (151.1) was that of the treatment with no phosphate application and no inoculation, and the highest (213.4) that of the treatment of inoculation with *P. fluorescens* and application rate of 75 kg/ha (Table 6).

Table 6. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on the number of pods per plant

Inoculation level / Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	151.06	152.86	159.60	157.20
25	177.40	175.93	184.86	175.93
50	184.80	190.86	194.93	188.53
75	180.53	176.20	213.40	194.80
100	179.73	179.67	184.53	185.53

Pod length: Only phosphate application had significant effects on pod length, and that the effects of inoculation, or the interaction effects of inoculation and phosphate application, on pod length were not significant (Table 1). In the inoculation treatments, the longest pods (4.6) belonged to the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens*, and the shortest to the treatment of inoculation with *P. putida* and to the control with 4.41 and 4.42 cm, respectively. Comparison of the means related to phosphate application showed that the shortest pods (4.05 cm) were those of the treatment in which phosphate was not applied (this pod length was statistically different from those of pods in the other treatments). The longest pods were observed in the treatment of applying phosphate at 75 kg/ha (and these pods were not significantly longer than pods in the treatments of applying phosphate 25, 50, or 100 kg/ha) (Table 7). Comparing the means of the interaction effects of inoculation and phosphate application indicated the longest pods (4.89) were observed in the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens* and no phosphate application, and the shortest (3.8 cm) in the treatment of inoculation with *P. putida* and no phosphate application. There were no significant difference between these pods of minimum length and those of the treatment with no inoculation and no phosphate application (in which the pods were 3.9 cm long) (Table 7).

Table 7. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on pod length

Inoculation level / Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	3.91	3.87	4.31	4.11
25	4.24	4.55	4.62	4.75
50	4.60	4.51	4.75	4.59
75	4.66	4.56	4.64	4.89
100	4.71	4.54	4.51	4.68

Number of seeds per pod: The results of analysis of variation indicated that the effects of phosphate solubilizing bacteria and those of phosphate application rate on number of seeds per pod were significant at the 5 and 1% probability levels, respectively. The interaction effects of inoculation and phosphate application on this trait were not significant (Table 1). In the inoculation treatments, the maximum number of seeds per pod (32.2) belonged to the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens*, and the minimum (28.2) to the treatment of inoculation with *P. putida*. This minimum number of seeds per pod was not significantly different from those of the treatment of inoculation with *P. fluorescens* and of the control, with 29.04 and 29.2 seeds per pod, respectively (Table 8). Comparison of the means related to phosphate application showed that the smallest number of seeds per pod (25.5) was that of the treatment with no phosphate application, and the largest (31.9) that of the treatment in which phosphate was applied at 75 kg/ha. This largest number was not significantly different from the number of pods per plant in the treatments of applying phosphate at 25, 50, or 100 kg/ha (Table 8). Comparing the means of the interaction effects of inoculation and phosphate application revealed that the highest number of seeds per pod (35.8) was observed in the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens* and application of phosphate at 75 kg/ha, and the lowest (24.9) in the treatment of inoculation with *P. putida* with no phosphate application. This lowest number was not significantly different from 25.1 (the number of seeds per pod in the treatment with no phosphate application and no inoculation) (Table 8).

Table 8. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on number of seeds per pod

Inoculation level / Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	25.18	24.92	26.55	25.41
25	29.59	27.80	28.30	32.30
50	30.30	28.35	29.16	35.04
75	30.66	30.68	30.66	35.80
100	30.64	29.44	30.50	32.54

Seed yield: The results of analysis of variation showed that the individual effects of inoculation on seed yield at the 5% probability level and of phosphate application rates at the 1% probability level, and the interaction effects of phosphate application and inoculation at the 5% probability level, were significant (Table 1). Comparing the means of inoculation effects on seed yield showed that the maximum seed yield (2610 kg/ha) belonged to the treatment of inoculation with P. fluorescens, which was not significantly different from that of the treatment of simultaneous inoculation with P. putida and P. fluorescens (2473 kg/ha). The minimum seed yield (2224 kg/ha) was that of the treatment that was not inoculated (Table 9). Comparing the means of the effects of phosphate application o seed yield indicated that the largest seed yield (2840 kg/ha) was that of the treatment of applying 75 kg/ha, and the smallest (1810 kg/ha) that of the treatment with no phosphate application (Table 9). Comparing the means of the interaction effects of phosphate application rate and inoculation revealed that the maximum seed yield (2980 kg/ha) was observed in the treatment of simultaneous inoculation with P. putida and P. fluorescens and application of phosphate at 75 kg/ha. This maximum seed yield was not statistically different from that of the treatment in which phosphate was applied at 75 kg/ha. The minimum seed yield (1480 kg/ha) belonged to the treatment of inoculation with P. putida and no phosphate application, which was not significantly different from that of the treatment in which seeds were not inoculated phosphate was not applied (which was 1600 kg/ha) (Table 9).

Table 9. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on seed yield

Inoculation level / Phosphate rate (kg/ha)	Control (no inoculation)	*P. putida*	*P. fluorescens*	Inoculation with both species
0	1600	1480	2030	2130
25	2230	2380	2490	2510
50	2150	2640	2860	2790
75	2650	2790	2940	2980
100	2490	2630	2730	1953

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