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Effects of phosphate solubilizing bacteria and mineral phosphorous levels on yield and yield components of canola Hyola 401 cultivar

F. Dehpouri[a], A. Vahedi[b,*], E. Yasari[c], O. Ghasemi Chepi[d], M.H. Haddadi[d]

[a]M.Sc. student, Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources, Islamic Azad University, Qaemshahr Branch, IRAN.
[b]Assistant Professor, the Islamic Azad University of Ghaemshahr.
[c]Assistant Prof, Department of Agricultural Science, Payame Noor University, I.R. of IRAN (Correponding author).
[d]Faculty Member, Agriculture Sciences and Natural Resources Research Center of Mazandaran.

*Corresponding author; Assistant Professor, the Islamic Azad University of Ghaemshahr.

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Split-plot experiment using the randomized complete block design with three replications was conducted at Dasht-e-Naz Agronomy Research Station in 2013 to study the effects of phosphate solubilizing bacteria and mineral phosphorous application on yield and yield components of canola. Treatments included four levels of bacteria (the control, Pseudomonas putida, Pseudomonas fluorescens, and simultaneous use of both bacterial species) in the main plots. Five levels of mineral phosphorous application (the control, 25, 50, 75, and 100 kg/ha of phosphorous in the form of concentrated superphosphate) were applied in the sub plots. Comparison of the means showed that phosphorous application had significant effects on all characteristics except plant height and cutting height of plants at harvest. Seed yields at 25, 50, and 75 kg/ha of phosphorous application were 963.8, 1006, and 1032 kg/ha, respectively (these differences were not statistically significant). The maximum seed yield (987.8 kg/ha) was obtained in the treatment of inoculation with P. fluorescens, but there was no statistically significant difference between this yield and that of the treatment of inoculation with both bacterial species (972.1 kg/ha). The minimum seed yield (852.4 kg/ha) was that of the treatment with no bacterial inoculation. Results also revealed that the level of phosphorous application and inoculation, and their interaction effects, had significant effects on number of pods of main stem and per plant. Under the interaction effects of phosphorous and bacteria, the minimum number of pods on the main stem (50.31) observed in the treatment in which no phosphorous was applied nor was any inoculation performed, and the maximum (66.77) in that of the treatment in which both bacterial species were used and 50 kg/ha of phosphorous was applied. Moreover, under the interaction effects of phosphorous and bacteria, the largest number of pods per plant (149.4) belonged to the treatment in which P. fluorescens was used and 75 kg/ha of phosphorous was applied.

1. 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). Klopper et al (1989) were able to increase potato growth under greenhouse conditions compared to the control by inoculation with two bacterial species of the genus Pseudomonas. Maximum nutrient absorption happens in inoculation with phosphate solubilizing bacteria because these bacteria cause morphological changes in root hairs and increase the number, thickness, and length of roots (Yanni et al., 1997).

It seems that symbiotic bacteria increase the level of root activity and that they secrete organic complexes that chelate micronutrients, thereby enabling host plants to absorb them. Zinc and manganese are essential elements for plants, and their deficiency usually happens in the early part of the growing season. Total soil zinc and manganese contents are sufficient to meet the needs of plants for many years, but many soil factors and conditions influence the availability of these elements so that only a small part of their total quantities in soils is available during each growing season (Marschner, 1995).

The role played by organic acids in solubilizing insoluble phosphates is attributed to reducing pH, chelating cations, and competing with phosphorous for occupying absorption sites in the soil. Moreover, it was reported that organic acids may form soluble complexes with metal ions such as calcium, aluminum, and iron bonded with phosphorous, thereby freeing phosphorous (Omar, 1998). 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 (Kapulink et al., 1985). Wheat phosphorous content increased considerably in the presence of Azotobacter and Pseudomonas striata (Almas and Saghir, 2005).

This research was conducted in Dasht-e-Naz (in Sari) with the purposes of studying the effects of phosphate solubilizing bacteria on the quantity of soil insoluble phosphorous absorption, to compare these effects with that of mineral phosphorous, and to investigate the possibility of replacing mineral phosphorous application with these bacteria at the final planting date of canola cultivar Hyola 401.

2. 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). Data were analyzed following the analysis of variance technique (ANOVA) and the mean differences were adjudged by Duncan's multiple range test.

3. Results and discussion

Results of ANOVA related to plant height and cutting height of plants at harvest showed that the levels of bacterial inoculation and phosphorous application did not have significant effects on these characteristics (Table 1). The interaction effects of phosphorous and bacteria on plant height were significant at the 1% probability level, but these effects were not significant on the cutting height of plants at harvest. Comparison of the means indicated the shortest plants (68.87 cm) belonged to the treatment with no phosphorous application (and these plants were shorter than those in treatments in which phosphorous was applied). Moreover, the shortest plants (71 cm) were observed in the treatment of no inoculation with P. fluorescens (and these plants were shorter than those in the treatments with bacterial inoculation) (Table 2).

The shortest plants under the interaction effects of phosphorous and bacterial inoculation (65.27 cm) belonged to the treatment of inoculation with P. putida with no phosphorous application, and the tallest (78 cm) in the treatment of inoculation with P. fluorescens and phosphorous application at 50 kg/ha (Table 3).

The results obtained related to number of lateral shoots revealed that the effects of phosphorous application and the interaction effects of phosphorous application and bacterial inoculation were significant at the 1% probability level, but that the levels of bacterial inoculation had no significant effects on this characteristic (Table 1). The largest number of lateral shoots (3.23) was observed in the treatment of applying 75 kg/ha of phosphorous, but this number was not significantly different from those obtained by applying phosphorous at 25 or 50 kg/ha. The smallest number of lateral shoots (2.68) belonged to the treatment in which inoculation was not performed, and the largest (3.62) to the treatment in which the two bacterial species were employed simultaneously (Table 2). The minimum number of lateral shoots (2.38) was produced under the interaction effects of no phosphorous application and no inoculation, and the maximum in the treatment of using both bacterial species and applying phosphorous at 75 kg/ha (Table 3).

The results related to number of pods on the main stem and per plant revealed that the level of phosphorous application and inoculation, and their interaction effects, had significant effects on these two characteristics at the 5, 1, and 5% probability level, respectively (Table 1). The maximum number of pods on the main stem (59.41) belonged to the treatment of applying 75 kg/ha of phosphorous (which was not significantly different from the corresponding numbers in the treatments of applying other levels of phosphorous). Moreover, the minimum number of pods on the main stem (54.36) was observed in the treatment of no phosphorous application and the maximum (61.85) in the treatment in which both bacterial species were used (Table 2). Under the interaction effects of phosphorous and bacteria, the smallest number of pods on the main stem (50.31) was that of the treatment in which no phosphorous was applied nor was any inoculation performed, and the largest (66.77) that of the treatment in which both bacterial species were used and 50 kg/ha of phosphorous was applied (Table 3). Moreover, the maximum number of pod per plant (133.9) was observed when 75 kg/ha of phosphorous was applied. The minimum number of pods per plant (122.5) was obtained when no inoculation was performed, and the maximum (134.9) in the treatment of inoculation with P. fluorescens (Table 2). Under the interaction effects of phosphorous and bacteria, the largest number of pods per plant (149.4) belonged to the treatment in which P. fluorescens was used and 75 kg/ha of phosphorous was applied.

Table 1
Analyze of variation of the data related to the studied characteristics.

Sources of Variation	**Degree of freedom**	**Mean Squares**			
		Plant height (cm)	**Plant cutting height at harvest(cm)**	**No. of lateral shoots**	**No. of pods on the main stem**
Replications	2	0.364 ns	5.102 ns	0.184 ns	42.187 ns
Phosphorous level (Factor A)	3	69.434 ns	2.245 ns	2.654**	213.567*
Factor A error	6	33.271	10.128 ns	0.137	24.407
Bacteria level (Factor B)	4	69.925**	0.755 ns	0.599**	101.666**
Interaction effects	12	20.648 ns	3.645 ns	0.035 ns	18.089*
Error	32	15.859	2.255	0.45	6.605
Coefficient of variation (%)	-	5.47	5.16	7.03	4.48

The symbols *, **, and ns stand for significant at the 5%, 1%, and not significant, respectively.

Table 2
Comparing Means of individual effects on the studied characteristics based on Duncan's multiple range test.

Treatments	**Plant height (cm)**	**Plant cutting height at harvest (cm)**	**No. of lateral shoots**	**No. of pods on the main stem**
Phosphorous (kg/ha)				
Zero	68.87 [b]	28.98 [a]	2.638 [c]	52.42 [b]
25	74.43 [a]	29.53 [a]	3.093 [ab]	57.42 [a]
55	73.92 [a]	28.96 [a]	3.078 [ab]	58.53 [a]
75	74.06 [a]	28.91 [a]	3.233 [a]	59.41 [a]
100	72.98 [a]	29.08 [a]	2.988 [b]	59.34 [a]
Bacteria				
putida	71.04 [a]	28.63 [a]	2.800 [b]	54.22 [b]
fluorescens	75.15 [a]	29.58 [a]	2.921 [b]	59.27 [a]
Both species	74.23 [a]	29.10 [a]	3.620 [a]	61.85 [a]

Means with similar letters in each column are not significantly different at the 5% probability level.

The results of the data on number of seeds per pod indicated that the effects of applying phosphorous and using bacteria on this characteristic were significant at the 5 and 1% probability levels, respectively, but that the interaction effects of phosphorous and bacteria were not significant (Table 1). The highest numbers of seeds per pod (22.37 and 22.55) belonged to the treatments of applying 75 kg/ha of phosphorous and of the simultaneous use of both bacterial species, respectively (Table 2). Under the interaction effects of phosphorous and bacteria, the lowest number of seeds per pod (17.63) was that of the control and the highest (25.06) that of simultaneous inoculation with both bacterial species and application of phosphorous at 75 kg/ha (Table 3).

Table 3
Comparing the means of the interaction effects of bacteria and phosphorous.

Treatments	Plant height (cm)	Plant cutting height at harvest (cm)	No. of lateral shoots	No. of pods on the main stem
Bacteria * phosphorous				
No bacteria * No phosphorous	67.01 bc	27.51 bc	2.380 h	50.31 h
No bacteria * phosphorous at 25 kg/ha	75.51 a	28.91 abc	2.660 g	55.44 efg
No bacteria * phosphorous at 50 kg/ha	71.07 abc	28.91 abc	2.707 fgh	52.27 gh
No bacteria * phosphorous at 75 kg/ha	67.15 bc	29.82 abc	2.963 defg	57.96 def
No bacteria * phosphorous at 100 kg/ha	74.25 ab	30.1 abc	2.707 fgh	55.81 efg
P. putida				
P. putida * no phosphorous	65.27 c	29.77 abc	2.427 h	52.36 gh
P. putida * phosphorous at 25 kg/ha	73.08 ab	30.05 abc	2.940 defg	54.27 fgh
P. putida * phosphorous at 50 kg/ha	70.28 abc	27.11 c	2.847 efg	54.09 fgh
P. putida * phosphorous at 75 kg/ha	75.74 a	27.72 abc	2.940 defg	54.13 fgh
P. putida * phosphorous at 100 kg/ha	70.82 abc	28.51 abc	2.847 efg	56.23 efg
P. fluorescens				
P. fluorescens * no phosphorous	71.57 abc	28.50 abc	2.427 h	54.46 fgh
P. fluorescens * phosphorous at 25 kg/ha	74.53 ab	29.69 abc	3.080 cdef	58.71 cdef
P. fluorescens * phosphorous at 50 kg/ha	78.07 a	30.53 a	3.127 cde	60.99 bcd
P. fluorescens * phosphorous at 75 kg/ha	77.00 a	29.99 abc	3.033 defg	59.97 cde
P. fluorescens * phosphorous at 100 kg/ha	74.57 ab	29.19 abc	3.940 d	62.02 bcd
Both bacterial species				
Both bacterial species * no phosphorous	71.63 abc	30.15 ab	3.320 bcd	52.36 gh
Both bacterial species * phosphorous at 25 kg/ha	74.62 ab	29.45 abc	3.693 ab	61.27 bcd
Both bacterial species * phosphorous at 50 kg/ha	76.25 a	29.31 abc	3.633 ab	66.77 a
Both bacterial species * phosphorous at 75 kg/ha	76.35 a	28.09 abc	3.993 a	65.58 ab
Both bacterial species *phosphorous at 100 kg/ha	72.29 abc	28.51 abc	2.460bc	63.29 abc

Means with similar letters in each column do not exhibit any significant differences at the 5% probability level.

Table 4
ANOVA of the data related to studied characteristics.

Source of Variation	Degree of freedom	Mean Squares			
		No. of pods per plant	Pod length(cm)	No. of seeds per pod	Seed yield (kg/ha)
Replications	2	677.262 ns	0.594 ns	45.210*	1481.867 ns
Phosphorous levels (A)	3	521.278 ns	0.500 ns	22.265*	54840.283**
Error in factor A	6	185.304	0.796 ns	4.291	1572.133
Bacterial level (factor B)	4	1169.833**	1.752**	36.467**	96978.942**
Interaction effects	12	39.811 ns	0.084 ns	2.431 ns	1748.908
Error	32	61.907	0.317	2.485	9065.379
Coefficient of variation	-	6.22	14.61	7.58	10.16

*, **, ns represent significant at 5%, 1%, and not significant, respectively.

Table 5
Comparing the means of individual and interaction effects on the studied characteristics based on Duncan's test.

Treatments	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Seed yield (kg/ha)
Phosphorous application (kg/ha)				
0	109.9 c	3.339 c	17.86 c	845 b
25	125.8 b	3.811 bc	20.65 b	963.8 a
50	133.7 a	4.402 a	21.50 ab	1032 a
75	133.9 a	3.953 ab	22.37 a	1006 a
100	129.2 ab	3.771 bc	21.55 ab	839.6 b
Bacterial inoculation				
No inoculation	122.3 a	3.672 a	20.50 b	852.4
P. putida	122.5 a	3.795 a	19.77 b	936.2 b
P. fluorescens	134.9 a	4.105 a	20.33 b	987.8 a
Both bacterial species	126.3 a	3.849 a	22.55 a	972.1 a

Means with similar letters in each column do not show significant differences at the 5% probability level.

Table 6
Comparison of the means related to the interaction effects of bacteria and phosphorous.

Treatments	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Seed yield (kg/ha)
Bacteria * phosphorous				
No bacteria * no phosphorous	105.7 f	3.071 d	17.63 ef	740.0 e
No bacteria * 25 kg/ha phosphorous	124.2 cde	3.637 abcd	20.72 bcd	860.0 bcde
No bacteria * 50 kg/ha phosphorous	129.4 bcd	4.257 abc	21.21 bc	953.0 abcd
No bacteria * 75 kg/ha phosphorous	126.4 bcd	3.764 abcd	21.47 bc	958.0 abcd
No bacteria * 100 kg/ha phosphorous	125.8 bcd	3.630 abcd	21.45 bc	751.0 e
P. putida * no phosphorous	107.0 f	3.382 bcd	17.45 ab	822.0 de
P. putida * phosphorous at 25 kg/ha	123.2 cde	3.720 abcd	19.46 f	962.0 abcd
P. putida * phosphorous at 50 kg/ha	133.6 bc	4.563 a	19.85 cdef	1026.0 abc
P. putida * phosphorous at 75 kg/ha	123.3 cde	3.627 abcd	21.48 bcdef	1004.0 abcd
P. putida * phosphorous at 100 kg/ha	125.3 bcd	3.680 abcd	20.61 bcde	867.0 bcde
P. fluorescens * no phosphorous	116.7 def	3.687 abcd	18.59 cdef	904.0 abcde
P. fluorescens * phosphorous at 25 kg/ha	132.7 bc	3.888 abcd	19.81 bcdef	1023.0 abc
P. fluorescens * phosphorous at 50 kg/ha	139.8 ab	4.470 ab	20.42 bcdef	1073.0 a
P. fluorescens * phosphorous at 75 kg/ha	149.4 a	4.453 ab	21.46 bc	1039.0 ab
P. fluorescens * phosphorous at 100 kg/ha	135.8 abc	4.028 abcd	21.35 bc	900. 0 abcde
Both bacterial species * no phosphorous	110.0 ef	3.214 cd	17.79 def	914.0 abcde
Both bacterial species * 25 kg/ha phosphorous	123.2 cde	3.999 abcd	22.61 ab	1010.0 abc
Both bacterial species * 50 kg/ha phosphorous	132.0 bcd	4.317 abcd	24.53 a	1074.0 a
Both bacterial species * 75 kg/ha phosphorous	136.4 abc	3.968.abcd	25.06 a	1022.0 abc
Both bacterial species * 100 kg/ha phosphorous	129.9 bcd	3.744 abcd	22.78 ab	840.3 cde

Means with similar letters in each column do not show significant differences at the 5% probability level.

Comparing the means of the effects phosphorous application had on seed yield indicated that maximum seed yield (1032 kg/ha) was achieved at 50 kg/ha of phosphorous (which was not significantly different from 1006 and 963.8 kg/ha obtained in the treatments of applying 75 and 25 kg/ha of phosphorous, respectively) (Table 1). The minimum seed yield (839.6 kg/ha) belonged to the treatment of applying 100 kg/ha of phosphorous (which was not significantly different from 845 kg/ha obtained in the treatment of no phosphorous application (Table 2).

Furthermore, comparing the means of the effects bacterial inoculation had on seed yield showed that the largest seed yield (987.9 kg/ha) was that of the treatment in which P. fluorescens was used (which was not significantly different with the yield of 972.1 kg/ha obtained in the treatment of simultaneous inoculation with both bacterial species) (Table 2).

Comparison of the means related to the interaction effects of phosphorous and bacteria indicated the highest seed yield (1075 kg/ha) was achieved in the treatment in which both P. putida and P. fluorescens were simultaneously used and phosphorous was applied at 50 kg/ha. This yield was not significantly different from the 1073 kg/ha obtained in the treatment of applying phosphorous at 50 kg/ha of phosphorous and using P. fluorescens). The lowest seed yield (740 kg/ha) was that of the treatment in which phosphorous was not applied and neither was inoculation performed. This yield was not significantly different from the 751 kg/ha obtained when no inoculation was performed but phosphorous was applied at 100 kg/ha (Table 3).

Besides making mineral soil elements available to plants, phosphate solubilizing bacteria act as nitrogen fixing agents, solubilize phosphorous and potassium, suppress pathogens, and increase yield through producing plant growth regulators (Sturz and Christie, 2003). Researchers found that adding Pseudomonas fluorescens increased yield of various crop plants (Kamal et al., 2008). Various species of Pseudomonas are effective in controlling pathogenic fungi through various mechanisms including production of siderophores, synthesis of antibiotics, production of hormones, increasing phosphorous absorption by plants, fixing nitrogen, synthesizing enzymes that regulate plant ethylene levels, stimulating growth, and increasing yield (Abdul-Jaleel et al., 2007).

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