

# Comparison of Response of Canola (*Brassica napus* L. cv Hyola 401) to Biofertilizer Inoculation in Optimal and Delayed Cropping Dates

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## Abstract

In order to compare the effects of phosphate solubilizing bacteria as biofertilizer and mineral phosphorous application in optimal date of sowing with delayed cropping date on canola (*Brassica napus* L.) growth and productivity, a split-plot experiment using randomized complete block design with three replications was conducted at Dasht-e-Naz Agronomy Research Station in 2014. Four levels of bacterial inoculation (*Pseudomonas putida*, *Pseudomonas fluorescens*, singly or in combination) were applied as main plots and five levels of mineral phosphorous application (0, 25, 50, 75, and 100 kg/ha of mineral phosphorous) were applied as sub plots. Canola plant were sown at two dates of sowing, one at optimal date of sowing (Sept. 30, 2014) and the other one month later as delayed cropping (Oct. 30, 2014). The results obtained showed that effects of bacterial inoculation on number of seeds per pod, number of pods on plant, and seed yield were significant at one percent probability level at both sowing dates. Moreover, rates of phosphate application had significant effects on all traits at one percent level. Comparing the means showed that application of mineral P resulted in substantial increase in seed yield. At optimal date of sowing, it was shown that while minimum seed yield obtained at control treatment (1600 kg/ha), the maximum (2980 kg/ha) obtained with interaction effects of application of both bacterial strains along with 75 kg/ha mineral P, having no statistically difference with that of *P. fluorescens* along with 75 kg/ha mineral P (2940 kg/ha). It was shown that delayed cropping resulted in decreasing canola growth which is reflected in seed yield and yield components. Minimum seed yield at control plot at first date of sowing (1600 kg/ha) decreased to 740 kg/ha in second date of sowing, showing 54% decrease. The maximum seed yield also decreased in delayed cropping, from 2980 kg/ha at Sept. 30, 2014 as compared to 1074 kg/ha at Oct. 30, 2014, showing a 64% decrease. The results obtained showed that an increase in P level, eventually enhanced the seed yield. This increasing trend continued until a threshold level (75 kg/ha of P), after which seed yield showed a declining fashion.

**Keywords:** canola, date of sowing, *Pseudomonas putida*, *Pseudomonas fluorescens*, phosphorous

## 1. Introduction

Choice of fertilizers and provision of conditions for optimum nutrient availability is of paramount importance that influences canola (*Brassica napus* L.) growth and yield, since canola plant is very efficient at taking up nutrients. 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). Using a judicious combination of chemical fertilizers as well as biofertilizers to obtain the best results can be strategic step. Reports are available on the use of biofertilizers in many crop plants in increasing crop yield and nutrient uptake. Positive effects of inoculation with *Azotobacter* and *Azospirillum* on canola (Yasari et al., 2008), wheat and mustard (P. Gupta & V. Gupta, 2006), mustard (Sharma et al., 1997) have already been reported. Yasari and Rafati Alashti (2013) observed that seed inoculation with *Pseudomonas fluorescens* and *putida* resulted in increased soybean seed yield compared to control. They reported that seed yield reached its maximum level when simultaneously was inoculated with both bacterial species. This maximum seed yield was accompanied by the maximum number of pods on the main stem, the highest number of pods per plant, and the largest 1000-seed weight. Under interaction effects of cultivar, phosphorous application, and bacterial inoculation, the maximum seed yield was obtained when seeds of the cultivar Telar were inoculated with *P. putida* and 50 kg/ha

of phosphorous were applied to the soil. Likewise Gleddie et al. (1993) reported that *Penicillium bilaji* increased the vegetative growth, P uptake, and grain yield of canola both with or without P fertilizer under P responsive conditions, most likely by solubilizing unavailable soil P. Almas and Saghir (2005) observed that phosphorous content increased considerably in the presence of *Pseudomonas striata* and *Azotobacter* in wheat seed. Attention has seldom been given to the nutrient requirement of the soil especially at optimum sowing dates and comparing this with delayed cropping, canola crops have suffered in most cases, either from lack or excess of these nutrients. Hence, the necessity to evaluate the best nutrient uptake for improving canola seed yield, quality and fortification in Mazandaran crops, based on soil test results. The present research study compared canola (*Brassica napus* L. cv Hyola 401) response to biofertilizer application inoculation in optimal and delayed cropping dates Dasht-e Naz in Sari.

## 2. Materials and Methods

In order to compare canola (*Brassica napus* L. cv Hyola 401) response to biofertilizer application inoculation in optimal and delayed cropping dates an experiment was conducted at Dasht-e-Naz Agronomy Research Station, Mazandaran, Iran in the cropping year 2014-2015. Canola plant have been sown at two dates of sowing, one at optimal date of sowing (Sept. 30, 2014) and the other one month later as delayed cropping (Oct. 30, 2014). The experimental site has an altitude of 13.5 m, longitude of 53°11' and latitude of 36°04'. The average annual rainfall at the station was 685 mm, and it has a temperate Caspian climate and maximum and minimum annual temperatures of 27.3 °C and 7.1 °C. The experiment was carried out in the split plot 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. 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). Results of tests carried out on these samples showed that the organic matter, the available phosphorous, and the available potassium contents of the soil were 2.51%, 6.7 ppm, and 297 ppm, respectively. The soil pH was 7.7 with loamy clay texture containing 37% clay, 26% silt, and 37% sand. 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). 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 number of seed per pod, number of pod in main stem and per plant, the length of pod, and the 1000-seed weight) were measured. Analysis of the variance was performed using the SAS statistical software, and comparison of the means of the data was performed on the basis of Duncan's multiple range test at the five percent probability level.

## 3. Results and Discussion

### 3.1 Number of Lateral Shoots

The results of analysis of variation showed that at first date of sowing (Sept. 30) only level of bacterial inoculation was significant on number of lateral shoots at 1% level (Table 1). The results obtained showed that number of lateral shoots increased with application of either treatments mineral P or phosphate solubilizing bacteria (PSB) in both sowing dates. At optimal date of sowing (first date), minimum number of lateral shoots (3.40) obtained at control treatments (neither application of mineral P nor bacterial inoculation) and the maximum number (4.46) at interaction effects of both bacterial species along with 25 or 75 kg/ha mineral P (Table 3). Analysis of variation at second date of sowing (Oct. 30) also showed that main effects of rates of mineral P and level of bacterial inoculation were significant on number of lateral shoots at 1% level (Table 2). At delayed cropping the minimum number of lateral shoots (2.38) obtained at control treatments and the maximum number (3.99) at interaction effects of both bacterial species along with 75 kg/ha mineral P (Table 2). Dehpouri et al. (2015) reported that maximum 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. They also observed that maximum number of lateral shoots was obtained at the simultaneous application of both the bacterial strains *P. putida* and *P. fluorescens* in canola plants. The results of present study were in agreement with those observed by Dehpouri et al. (2015) and Freitas et al. (1997).

Table 1. Analysis of variation of the data related to the studied traits at the first date of sowing

Sources of Variation	Degree of Freedom	Mean Squares					
		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	0.002	75.266	951.122	1.604	92.266**	1481.867 ns
Rate of phosphate	3	0.487 <sup>ns</sup>	216.886*	537.878*	0.155	45.439*	54840.283**
Error	6	0.302	34.560	391.629	0.411	8.757	1572.133
Level of bacterial inoculation	4	1.161**	138.526**	2528.918**	0.792**	74.423**	96978.942**
Interaction effects	12	0.063 <sup>ns</sup>	0.063 <sup>ns</sup>	123.512 <sup>ns</sup>	0.691 <sup>ns</sup>	4.962 <sup>ns</sup>	1748.908*
Error	32	0.052	0.052	152.103	1.630	5.072	9065.379
Coefficient of variation (%)	-	5.64	5.64	6.87	5.01	7.58	10.16

Note. ns, \*, \*\* refer to non significant, significant at five and one percent probability levels, respectively.

Table 2. Analysis of variation of the data related to the studied traits at the second date of sowing

Source of Variation	Degree of freedom	Mean Squares					
		No. of lateral shoots	No. of pods on the main stem	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Seed yield (kg/ha)
Replication	2	0.184 ns	42.187 ns	677.262 ns	0.594 ns	45.210*	2020.07 ns
Rate of phosphate	3	2.654**	213.567*	521.278 ns	0.500 ns	22.265*	5481.12**
Error	6	0.137	24.407	185.304	0.796 ns	4.291	1325.45
Level of bacterial inoculation	4	0.599**	101.666**	1169.833**	1.752**	36.467**	869.112**
Interaction effects	12	0.035 ns	18.089*	39.811 ns	0.084 ns	2.431 ns	456.11 ns
Error	32	0.45	6.605	61.907	0.317	2.485	9065.379
Coefficient of variation	-	7.03	4.48	6.22	14.61	7.58	10.16

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

Table 3. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on No. of lateral shoots

Phosphate rate (kg/ha)	Inoculation level	Control (no inoculation)	<i>P. putida</i>	<i>P. fluorescens</i>	Inoculation with both species	Average of Phosphate rate
		First date of sowing (Sept. 30)	0	3.4 c	3.46 c	3.46 c
	25	3.8 c	4.20 bc	4.40 ab	4.46 a	4.21
	50	3.86 c	4.06 bc	4.46 a	4.33 ab	4.18
	75	4.23 bc	4.20 abc	4.33 ab	4.20 abc	4.24
	100	3.86 c	4.06 bc	4.20 abc	4.46 a	4.15
Average of bacterial inoculation		3.83	4	4.17	4.23	
Second date of sowing (Oct. 30)	0	2.38 h	2.42 h	2.42 h	3.32 bcd	2.63
	25	2.66 g	2.94 defg	3.08 cdef	3.69 ab	3.09
	50	2.70 fgh	2.84 efg	3.12 cde	3.63 ab	3.07
	75	2.96 defg	2.94 defg	3.03 defg	3.99 a	3.23
	100	2.70 fgh	2.84 efg	3.94 a	2.46 bc	2.98
Average of bacterial inoculation		2.68	2.79	3.11	3.41	

Note. Values within a column followed by same letter are not significantly different at Duncan ( $p \leq 0.05$ ).

### 3.2 Number of Pods on the Main Stem

Comparison of the means revealed that number of pods on main stem increased with application of mineral P and bacterial inoculation. In both factors minimum number of pods on the main stem obtained at control

treatments whereas the maximum number obtained at higher level of application of mineral P or bacterial inoculation (Table 4). Numbers of pods are the most important yield components affecting seed yield, which is already described by several researchers (Shukla et al., 2002; Khalid et al., 2004; Yasari et al., 2009). Comparing the means of the interaction effects of phosphate application and inoculation at first date of sowing showed that minimum number of pods on the main stem (71.86) was at control plot, and the maximum (87.53) at the treatment in which seeds were inoculated with both bacterial along with 25 kg/ha of mineral P application (Table 4). At the second date of sowing however, the minimum number of pods on the main stem (50.31) was at control and the maximum (66.77) belonged to the treatment in which both bacterial species were applied along with 50 kg/ha of mineral phosphorous. The results of the present investigation was fairly comparable with those obtained by Saber et al. (2013) who suggested an increased number of pods in canola plant by PGPR.

Table 4. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on number of pods on the main stem

Phosphate rate (kg/ha)	Inoculation level					Average of Phosphate rate
	Control (no inoculation)	<i>P. putida</i>	<i>P. fluorescens</i>	Inoculation with both species		
First date of sowing (Sept. 30)	0	71.86 f	74.80 ef	78.67 cde	74.80 ef	74.88
	25	79.20 cde	77.53 de	83.86 abc	87.53 a	82.03
	50	74.66 ef	77.26 de	87.13 ab	85.86 ab	81.23
	75	82.80 bc	77.33 de	85.66 ab	86.06 ab	82.96
	100	79.73 cd	80.33 cd	86.60 ab	83.26 abc	82.98
Average of bacterial inoculation		77.65	77.45	84.66	83.50	
Second date of sowing (Oct. 30)	0	50.31 h	52.36 gh	54.46 fgh	52.36 gh	52.37
	25	55.44 efg	54.27 fgh	58.71 cdef	61.27 bcd	57.42
	50	52.27 gh	54.09 fgh	60.99 bcd	66.77 a	58.53
	75	57.96 def	54.13 fgh	59.97 cde	65.58 ab	59.41
	100	55.81 efg	56.23 efg	62.02 bcd	63.29 abc	59.33
Average of bacterial inoculation		51.35	54.21	59.23	61.85	

Note. Values within a column followed by same letter are not significantly different at Duncan ( $p \leq 0.05$ ).

### 3.3 Number of Pods per Plant

The number of pods per plant increased with application of mineral P as well as PSB. The maximum number obtained at the treatment of higher levels of P application either mineral or bacterial (Table 5). Comparing the means of the interaction effects of phosphate application and inoculation at first date of sowing showed that the minimum number of pods in plant (151.06) was at control plot, and the maximum (213.4) at the treatment in which seeds were inoculated with *P. fluorescens* at present of 75 kg/ha of mineral P application (Table 5). At the second date of sowing however, the minimum number of pods on plant (105.7) was at control and the maximum (149.4) belonged to the treatment in which *P. fluorescens* was applied along with 75 kg/ha of mineral P. These observations were in conformity with those observed Saber et al. (2013) and Freitas et al. (1997) in their study on effects of PGPR and PSB on canola, respectively.

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

Phosphate rate (kg/ha)	Inoculation level	Control	<i>P. putida</i>	<i>P. fluorescens</i>	Inoculation with	Average of
		(no inoculation)			both species	Phosphate rate
First date of sowing (Sept. 30)	0	151.06 e	152.86 e	159.60 de	157.20 e	155.18
	25	177.40 bcd	175.93 bcde	184.86 b	175.93 bcde	178.53
	50	184.80 b	190.86 ab	194.93 ab	188.53 b	189.78
	75	180.53 b	176.20 bcd	213.40 a	194.80 ab	191.23
	100	179.73 bc	179.67 bc	184.53 bc	185.53 b	182.21
Average of bacterial inoculation		174.70	174.98	187.46	180.40	
Second date of sowing (Oct. 30)	0	105.7 f	107.0 f	116.7 def	110.0 ef	109.85
	25	124.2 cde	123.2 cde	132.7 bc	123.2 cde	125.82
	50	129.4 bcd	133.6 bc	139.8 ab	132.0 bcd	133.7
	75	126.4 bcd	123.3 cde	149.4 a	136.4 abc	133.87
	100	125.8 bcd	125.3 bcd	135.8 abc	129.9 bcd	129.2
Average of bacterial inoculation		122.3	122.48	134.88	126.3	

Note. Values within a column followed by same letter are not significantly different at Duncan ( $p \leq 0.05$ ).

### 3.4 Pod Length

The results obtained showed that pod length also was affected with application of mineral P as well as PSB. The minimum pod length obtained at control treatment at both dates of sowing (Table 6). Comparing the means of the interaction effects of phosphate application and inoculation at first date of sowing showed the minimum pods length (3.87 mm) with *P. putida* and no mineral P application. The maximum pods length (4.89 mm) at the treatment in which seeds were inoculated with both species inoculation along with 75 kg/ha of mineral P (Table 6). At the second date of sowing it was shown that the minimum pods length (3.07 mm) was at control and the maximum (4.45 and 4.47 mm) belonged to *P. fluorescens* with 75 and 50 kg/ha of mineral P. Similar observations have already been seen by Saber et al. (2013) and Freitas et al. (1997) in their study on effects of PGPR and PSB on canola, respectively.

Table 6. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on pod length

Phosphate rate (kg/ha)	Inoculation level	Control	<i>P. putida</i>	<i>P. fluorescens</i>	Inoculation with	Average of
		(no inoculation)			both species	Phosphate rate
First date of sowing (Sept. 30)	0	3.91 d	3.87 d	4.31 abcd	4.11 cd	4.05
	25	4.24 bcd	4.55 abc	4.62 abc	4.75 ab	4.54
	50	4.60 abc	4.51 abc	4.75 ab	4.59 abc	4.61
	75	4.66 abc	4.56 abc	4.64 abc	4.89 a	4.69
	100	4.71 ab	4.54 abc	4.51 abc	4.68 abc	4.61
Average of bacterial inoculation		4.42	4.41	4.57	4.61	
Second date of sowing (Oct. 30)	0	3.07 d	3.38 bcd	3.68 abcd	3.21 cd	3.33
	25	3.63 abcd	3.72 abcd	3.88 abcd	3.99 abcd	3.81
	50	4.25 abc	4.56 a	4.47 ab	4.31 abcd	4.40
	75	3.76 abcd	3.62 abcd	4.45 ab	3.96 abcd	3.95
	100	3.63 abcd	3.68 abcd	4.02 abcd	3.74 abcd	3.77
Average of bacterial inoculation		3.67	3.79	4.10	3.84	

Note. Values within a column followed by same letter are not significantly different at Duncan ( $p \leq 0.05$ ).

### 3.5 Number of Seeds per Pod

The results obtained showed that mineral P or bacterial inoculation increased the number of seeds per pod in both dates of sowing. The minimum number of seeds per pod obtained at control plots (25.18) and the maximum number (35.8) at the conjunctive application of 75 kg/ha mineral P and both bacteria strains at the first date of sowing (Table 7). Comparing the means of the interaction effects of inoculation and phosphate application at second date of sowing revealed that the maximum number of seeds per pod (25.06) was observed in the treatment of simultaneous inoculation with *P. putida* and *P. fluorescens* and application of phosphate at 75 kg/ha, and the minimum (17.45) in the treatment of inoculation with *P. putida* with no phosphate application. Numbers of seeds per pod are also considered to be important yield components affecting seed yield (Shukla et al., 2002; Khalid et al., 2004; Yasari et al., 2009). Similar observations were already made by Saber et al. (2013) in their investigation on effects of PGPR on rapeseed.

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

Phosphate rate (kg/ha)	Inoculation level					
	Control (no inoculation)	<i>P. putida</i>	<i>P. fluorescens</i>	Inoculation with both species	Average of Phosphate rate	
First date of sowing (Sept. 30)	0	25.18 c	24.92 c	26.55 bc	25.41 c	25.51
	25	29.59 abc	27.80 bc	28.30 bc	32.30 ab	24.98
	50	30.30 abc	28.35 bc	29.16 abc	35.04 ab	30.71
	75	30.66 abc	30.68 abc	30.66 abc	35.80 a	31.95
	100	30.64 abc	29.44 abc	30.50 abc	32.54 ab	30.78
Average of bacterial inoculation		29.27	28.24	29.03	32.21	
Second date of sowing (Oct. 30)	0	17.63 ef	17.45 ab	18.59 cdef	17.79 def	17.86
	25	20.72 bcd	19.46 f	19.81 bcdef	22.61 ab	20.65
	50	21.21 bc	19.85 cdef	20.42 bcdef	24.53 a	21.50
	75	21.47 bc	21.48 bcdef	21.46 bc	25.06 a	22.36
	100	21.45 bc	20.61 bcde	21.35 bc	22.78 ab	21.59
Average of bacterial inoculation		20.49	19.77	20.32	22.55	

Note. Values within a column followed by same letter are not significantly different at Duncan ( $p \leq 0.05$ ).

### 3.6 Seed Yield

The results of analysis of variation showed that at first date of sowing (Sept. 30, 2014) the individual effects of inoculation and its interaction effects with phosphate application on seed yield at the 5% probability level and of phosphate application rates at the 1% probability level were significant at both dates of sowing (Table 1). Comparing the means showed that application of mineral P resulted in substantial increase in seed yield. It was shown that while minimum seed yield obtained at control treatment (1600 kg/ha), maximum seed yield (2980 kg/ha) obtained at interaction effects of application of both bacterial strains along with 75 mineral P, having no statistically difference with that of *P. fluorescens* along with 75 mineral P (2940 kg/ha) (Table 8). The results obtained showed that an increase in P level, eventually enhanced the seed yield. This increasing trend continued until a threshold level (75 kg/ha of P), after which seed yield showed a declining fashion. Similar observations have been made by Baradari et al. (2013) in their examination on canola; Yasari and Rafati Alashti (2013) in their investigation on soybean and Ebrahimi Chamani et al. (2014) in their experiment on rice. It was also observed that applying higher rates of phosphorous together with inoculating seeds with bacteria resulted in lower seed yields. This could signify the antagonistic effects of bacterial inoculation with higher rates of phosphorous application. At the second date of sowing (Oct. 30, 2014) however, results of analysis of variation showed that the individual effects of inoculation and its interaction effects with phosphate application on seed yield at the 5% probability level and of phosphate application rates at the 1% probability level were significant at both dates of sowing (Table 2). Comparison of the means related to the interaction effects of phosphorous and bacteria indicated the maximum seed yield (1074 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 740 kg/ha obtained when no inoculation was performed but phosphorous was applied at 100 kg/ha (Table 8). It was shown that delayed cropping resulted in decreasing canola growth which is reflected in seed yield and yield components. Minimum seed yield at control plot at first date of sowing (1600 kg/ha) decreased (to 740 kg/ha) in second date of sowing, showing 54% decrease. The maximum seed yield also decreased in delayed cropping, from 2980 kg/ha at Sept. 30 2014 to 1074 kg/ha at Oct. 30, 2014, showing a 64% decrease. 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 & Christie, 2003). Researchers found that adding *Pseudomonas fluorescens* increased yield of various crop plants. These results are in agreement with those obtained by Yasari and Rafati Alashti (2013) in their research work on soybean crop. They reported that the *pseudomonas* species activity declined at the higher levels of mineral P availability, pointing the antagonistic effects of bacterial activity in higher levels of phosphorous availability. Similar observation have also been made by Tran et al. (2006), and Aris, Rika, and Giyanto (2011) in their study on effects of *Pseudomonas* species on soybean plant. Soleimanzadeh and Ghooshchi (2013) reported that high inputs cropping system was the most productive treatment but organic cropping system with biofertilizers was the most economical treatment with respect to increasing net profit in their study on maize. Winarso et al. (2011) found that the simultaneous use of humic acid and strains of the species of *P. putida* increased soil pH and, hence, raised the availability of phosphorous (and improved other soil features). They also reported that the simultaneous employment of humic acid and strains of *P. putida* increased seed yield. The results of the present study was in conformity with those obtained by Burd et al. (2000) who reported that PGPR might improve plant growth and productivity by synthesizing phytohormones, enhance the general availability of nutrients. It can be therefore concluded that instead of application of higher rates of mineral phosphorous without application of PSB, a combined application of phosphate solubilizing bacteria and optimum levels of mineral P can be more effective in terms of canola crop healthy growth and optimal productivity. These results definite the previous finding who reported growth and yield in various crop plants by microbial inoculation (Biswas et al., 2000; Fallik & Okon, 1988; Marcos et al., 1995; Naveed et al., 2008).

Table 8. Comparison of the means of the individual and interaction effects of phosphate and inoculation levels on seed yield

Phosphate rate (kg/ha)	Inoculation level					
	Control (no inoculation)	<i>P. putida</i>	<i>P. fluorescens</i>	Inoculation with both species	Average of Phosphate rate	
First date of sowing (Sept. 30)	0	1600 jk	1480 k	2030 gh	2130 hi	1810
	25	2230 i	2380 fg	2490 f	2510 ef	2402
	50	2150 hi	2640 cde	2860 ab	2790 bcd	2610
	75	2650 cde	2790 bcd	2940 ab	2980 a	2840
	100	2490 f	2630 de	2730 bede	1953 i	2450
Average of bacterial inoculation		2224	2384	2610	2472	
Second date of sowing (Oct. 30)	0	740.0 e	822.0 de	904.0 abcde	914.0 abcde	845
	25	860.0 bcde	962.0 abcd	1023.0 abc	1010.0 abc	963.75
	50	953.0 abcd	1026.0 abc	1073.0 a	1074.0 a	1031.5
	75	958.0 abcd	1004.0 abcd	1039.0 ab	1022.0 abc	1005.75
	100	751.0 e	867.0 bcde	900.0 abcde	840.3 cde	839.75
Average of bacterial inoculation		852.4	936.2	987.8	972.06	

Note. Values within a column followed by same letter are not significantly different at Duncan ( $p \leq 0.05$ ).

#### 4. Conclusion

In general it appears that delayed cropping resulted in decreasing canola growth which is reflected in seed yield and yield components. Seed yield at control plot at first date of sowing decreased in second date of sowing, showing 54% decrease. Application of *P. putida* and *P. fluorescens* can be effective in terms of canola growth augmentation. The effects of such biofertilizer reflects on different aspects of canola growth, yield and yield attributing characteristics. It was also shown that simultaneous application of both *pseudomonas* strains was more effective comparing to their individual application. It was observed that applying maximum level of

mineral phosphorous together with inoculating seeds with bacteria resulted in lower seed yields. This could signify the antagonistic effects of bacterial inoculation with higher rates of phosphorous application. Therefore it can be recommended that instead of application of exceeding rate of mineral P, which can cause retarding effects on other mineral nutrients especially micronutrients such as Zn, Fe, Mn, additional biofertilizers application such as PSB could be more effective and beneficial. In the other word it can postulated that instead of application of exceeding rates of mineral phosphorous without application of PSB, a combined application of phosphate solubilizing bacteria and optimum levels of mineral P can be more effective in terms of canola crop healthy growth and optimal productivity.

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