

## INTERACTION OF *PSEUDOMONAS FLUORESCENCE* BACTERIA AND PHOSPHORUS ON THE QUANTITATIVE AND THE QUALITATIVE YIELD OF RAPESEED (*BRASSICA NAPUS* L.) CULTIVARS

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**Abstract.** Canola (*Brassica napus* L.) is a very important crop. Hence, the effects of chemical and biological fertilizer on canola oil production and nutrient uptake, under calcareous conditions, were evaluated in a field experiment. The experiment was carried out in factorial in a randomized complete blocks design with three replications in Babolsar, Iran in 2010 and 2011. The bacterial inoculation factor comprised the following five levels: *Pseudomonas fluorescence* I (PSf<sub>1</sub>), *Pseudomonas fluorescence* II (PSf<sub>2</sub>), *Pseudomonas putida* I (PSp<sub>1</sub>), *Pseudomonas putida* II (PSp<sub>2</sub>), and control treatment (PS<sub>0</sub>) The triple superphosphate fertilizer factor comprised the consumption of triple superphosphate fertilizer based on the soil test (P<sub>1</sub>), the consumption of triple superphosphate fertilizer based on 25 % less than the soil test (P<sub>2</sub>), the consumption of triple superphosphate fertilizer based on 50 % less than the soil test (P<sub>3</sub>), and control treatment (P<sub>0</sub>) as well as the cultivars Hayula 401 and Sarigol. Results showed that the use of growth-stimulating bacteria had increased the seed yield, biologic yield, 1,000-seed weight and shoot, and grain phosphorus concentration of canola cultivars. The highest seed yield belonged to the *Pseudomonas putida* II bacteria, with an average of 3,587 kg ha<sup>-1</sup> and 17.4 % edge in comparison to the control group. The highest seed yield, with an average of 3,711 kg ha<sup>-1</sup>, which showed a 30 % increase in comparison to the control treatment, was obtained in the treatment of 25 % less phosphorus than the soil test. In the most investigated qualities, Hayula 401 excelled Sarigol, and the consumption of biologic fertilizers, as the complements of chemical fertilizers, increased the yield and yield components, and improved the phosphorus content of the leaf and the grain.

**Keywords:** biofertilizer, inoculation, canola, nutrient uptake, oil, phosphorus-solubilizing bacteria, sustainable agriculture

### Introduction

Rapeseed (*Brassica napus* L.) is one of the most important oilseeds in the world and the most important crop species of the *Brassica* type (Rosas et al., 2006). After soybean and oil palm, canola is the third highest source of oil production (Salimpour et al.,

2012). Despite this product's importance in the world as rich source of oil and protein, its yield in the Mazandaran Province is affected by special situations of water logging and phosphorus fixation in calcareous soil. As a result, nitrogen and phosphorus are two elements that limit canola yield in Mazandaran Province, Iran. Phosphorus is the second important key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement. Although abundant in soils, in both organic and inorganic forms, the availability of phosphorus is restricted as it occurs mostly in insoluble forms (Sharma et al., 2013). Local farmers normally use 100 to 150 kg of triple superphosphate fertilizer or ammonium phosphate; only 20–25 % of phosphorus is consumed by the plant due to phosphorus fixation in the soil (Sharma et al., 2013), which, using much chemical input, contributes to high energy consumption and greenhouse gas emissions (Pazouki et al., 2017). According to the reports presented in a short period of time, two-thirds of the used phosphate fertilizers get inaccessibly fixated in the soil (Afzal et al., 2005). Some of the most important biological fertilizers include phosphate-solvent bacteria. The release of phosphorus from the insoluble and the fixated forms by phosphate-solvent bacteria is relevant to the amount of accessible phosphorus in the soil and its transfer to the plant (Khan and Joergesen, 2009). Microorganisms increase the accessibility of plants to phosphorus by mineralizing organic phosphorus in soil through solving deposited phosphate (Chen et al., 2006; Kang et al., 2002; Pradhan and Sukla, 2005). According to reports, phosphate-solvent bacteria can constitute up to 88 % of all phosphate-solvent microorganisms (Falah, 2006). The use of phosphate-solvent microorganisms can increase crop yield up to 70 % (Verma, 1993). According to studies, phosphate-solvent bacteria, along with other plant-growth-promoting rhizobacteria, decreased the use of phosphate fertilizers up to 50 % without any significant decrease in crop production (Jilani et al., 2007; Yazdani et al., 2009). This is a good perspective toward the sustainable production of crops. The fixation or the mineralization of soil-solvable and inorganic phosphate chemical fertilizers constitutes an important factor for the fertilizer's inaccessibility to the plant (Dey, 1988). The *Pseudomonas* type of bacteria is especially important because of its expanded distribution in soil, and its ability to colonize the rhizosphere of many plants and to produce a great range of metabolites. One of the most important growth-promoting characteristics of plants is their ability to solve phosphate, which has been reported by researchers (Rashid et al., 2004; Ramezanpour, 2009).

Other study results showed that seed and straw yields, and the nitrogen uptake of canola were significantly increased by bacterization. Besides nitrogen, the uptake of other nutrients (Fe and Mn) was also increased by these microorganisms. In general, pyrite amendment along with bacterization improved yields and nutrient uptake in canola and wheat over other treatments without pyrite addition (Joseph et al., 2014). Both bacterial and fungal strains exhibiting P solubilizing activity are detected by the formation of a clear halo (a sign of solubilization) around their colonies. Production of a halo on a solid agar medium should not be considered the sole test for P solubilization. When colonies grow without a halo after several replacements of the medium, an additional test in liquid media to assay P dissolution should be performed and the few isolates that are obtained after such rigorous selection should be further tested for the abundant production of organic acids; isolates complying with these criteria should be tested on a model plant as the ultimate test for potential P solubilization (Bashan et al., 2013). Madani et al. (2011) revealed that statistical analyzes showed that PSB3 treatment was the best treatment for seed yield increase. The highest rate of seed yield

(9.9 t/ha) was recorded in dual fertilizing applied both in sowing time and after the overwintering stage of the rosette. The interaction effects of phosphorus-solubilizing bacteria and ammonium phosphate fertilizer application did not have a significant effect on plant height, biomass yield, the number of silique per plant, seed oil percentage, and seed yield. The interaction effects of phosphorus-solubilizing bacteria and the application of ammonium phosphate fertilizer were significant for phosphorus content in plant tissues.

The effect of the mentioned bacteria in increasing the function of plants, such as rice and canola, has been reported (Ramezanpour, 2010; Yasari and Patwardhan, 2007). Different results of studies have shown that the use of strains of *P. fluorescence* and *P. putida* have lengthened the roots of the shoot in canola and tomato, and have also increased the function in wheat, rice, and sugar beet (Egamberdiyeva et al., 2003; Dobbelaere et al., 2002; Rodriguez and Fraga, 1999). Yasari et al. (2008) reported that the inoculation of *Azotobacter* and *Spirillum* had increased the amount of canola oilseed. The purpose of this research was to investigate the effects of using different species of *P. fluorescent* as the solvent bacteria of the soil's insoluble phosphate on the function and phosphorus content of the leaves and the seeds of the canola cultivars Hayula and Sarigol. The results of previous studies were performed mostly under greenhouse conditions or the bacteria's individual effects on crop yield (Salimpour et al., 2012). This research investigated the effects of the inoculation of different types with different amounts of solving power and different amounts of triple superphosphate fertilizer on canola in the farm.

## Materials and methods

This farm experiment was performed in factorial in the form of randomized complete blocks in Babolsar between 2010 and 2011. The used *Pseudomonas* bacteria were supplied and chosen from the superior *Phosphorus solvent strains* in the previous studies of the fourth writer (Ramezanpour, 2009; 2011).

The main bacterial factor consists of five bacterial levels: *Pseudomonas fluorescence* I (PSf<sub>1</sub>), *Pseudomonas fluorescence* II (PSf<sub>2</sub>), *Pseudomonas putida* I (PSp<sub>1</sub>), *Pseudomonas putida* II (PSp<sub>2</sub>), and control treatment without bacteria (PS<sub>0</sub>). The fertilizer factor consists of four levels of triple superphosphate fertilizer; these include consuming fertilizer based in soil test (P<sub>1</sub>), 25 % less than the soil test (P<sub>2</sub>), 50 % less than the soil test (P<sub>3</sub>), and the level of control treatment (P<sub>4</sub>), and the cultivars Hayula 401 and Sarigol. It was performed in three replications on a farm in Babolsar, Mazandaran Province, Iran. The triple superphosphate fertilizer was consumed in four levels of 200, 150, 100, and 0 kg ha<sup>-1</sup>. According to the physical and the chemical analyzes of the soil laboratory, the clay loam soil has a pH of 7.4, total nitrogen of 0.12 %, organic material of 1.46 %, accessible phosphorus of 6.5 mg kg<sup>-1</sup>, and accessible potassium of 190 mg kg<sup>-1</sup>. The urea fertilizers were added in a quantity of 200 kg ha<sup>-1</sup> and the potassium sulfate was added in a quantity of 150 kg ha<sup>-1</sup> to the soil in the following three steps: before planting, at the end of tillering, and during the flowering stages. The area of each plot was 15 m<sup>2</sup>, including nine lines of planting, with a distance of 30 cm and a length of 5 m. The distance between the plants was determined to be 3–5 cm. The seeds were inoculated for 24 h in a temperature range of 24 ± 2 °C with inoculation liquids; they were placed to be dried in fresh air for two hours. In order to supply the inoculation liquid, bacteria were cultivated for 48 h in

nutrient Agar in at 24 °C. The population of the isolate was almost 108 mm of suspension. To inoculate the seeds, 7 mm of inoculation liquid was used for every 100 seeds. The seeds were poured in sterilized containers and immersed in inoculation liquid for 24 h. The seeds used in control treatment, like in bacterial treatment, bloomed in sterilized water and were then cultivated in experimental plots specific to each treatment. The data collected from this experiment includes phosphorus concentration in the shoot and the seed, seed yield, biological yield, 1,000-seed weight, the number of pods per plant, the number of seeds per pod, and the length of the pod during harvest. The measurement of the leaf and the seed phosphorus concentration was performed by the Olson method. The results of this experiment were statistically analyzed by the MSTATC software and the data mean was compared with Duncan's multiple range tests.

## Results and discussion

### Quantitative trait

The results in *Table 1* showed that in bacteria and phosphorous treatment, plant height, the height of the first pod, the number of pods per plant, the number of grains per pod, 1,000-seed weight, seed yield, biological yield, and harvest index were significant at the 5 % probability level. In addition, the cultivar effect and the interaction of year with cultivar were significant at the 5 % probability level for all the traits that have been described (*Table 1*). Interaction of bacteria × cultivar, year × phosphorous, bacteria × phosphorous, cultivar × phosphorous, and triple interaction had a significant effect on all the traits except pod length at the 5 % probability level; the number of grains per pod had no significant effect on the interaction of cultivar × phosphorous (*Table 1*).

**Table 1.** Mean square of quantities and qualities yield of rapeseed cultivars *pseudomonas fluorescence* bacteria and amounts of phosphorus

S.O.V.	DF	Plant height	Height of first pod	Pod length	Number of pod per plant	Number of grain per pod	1000-seed weight	Seed yield	Biological yield	Harvest index
Year (Y)	1	758.78	419.50	24.31	5927.22	2674.00	3.23	225190.10	27434.82	392.91
Error	4	117.97	3359.17	0.38	13343.51	36.19	0.24	1776481.03	11587492.86	94.04
Bacteria (B)	4	84.45*	4755.11*	0.07	4317.55*	43.74*	0.45*	2129631.30*	19573442.22*	11.82*
Y×B	4	49.91*	2922.23*	0.12	2983.92*	53.14*	0.20*	728064.62*	7210592.70*	102.86*
Cultivar (C)	1	10494.83*	20938.41*	15.87*	23540.22*	685.13*	10.78*	1785892.54*	72994334.02*	645.83*
Y×C	1	6040.67*	11213.50*	8.35*	62072.02*	45.50*	3.78*	9664.70*	365820835.27*	954.49*
B×C	4	150.06*	2646.04*	0.12	7586.11*	25.78*	0.18*	38579.47*	6254873.23*	33.78*
Phosphorous (P)	3	891.86*	3455.81*	0.11	17906.06*	23.78*	1.32*	8818923.84*	86008864.86*	24.00*
Y×P	3	233.14*	3123.34*	0.14	8543.96*	55.65*	0.26*	110139.98*	873928.16*	9.20*
B×P	12	83.33*	2870.01*	0.27	4433.21*	46.37*	0.13*	290189.86*	3268713.8*	7.01*
C×P	3	169.17*	1384.87*	0.04	3907.91*	0.50	0.04*	35721.55*	1202114.63*	3.99*
B×C×P	12	97.80*	2544.31*	0.33	6740.89*	93.62*	0.07*	14076.18*	1049884.93*	1.78*
Error	187	92.15	3003.76	0.18	5419.48	1198.81	0.13	87187.54	4748429.00	19.71
CV. (%)	-	6.24	5.98	7.43	40.19	13.11	10.03	8.84	15.95	17.64

\*\* and \*, respectively, significant in 1 % and 5 % level

The result of the main mean comparison showed that pod length, seed yield, and harvest index in the first year were, respectively, 11.45, 5.87, and 10.74 % more than the second year; the number of grains per pod and the 1,000-seed weight in the second year were, on the other hand, 41.77 and 6.94 %, respectively, more than the first year (*Table 2*).

**Table 2.** Mean comparison of quantities and qualities yield of rapeseed cultivars *pseudomonas fluorescence* bacteria and amounts of phosphorus

S.O.V.	Plant height (cm)	Height of first pod (cm)	Pod length (cm)	Number of pod per plant	Number of grain per pod	1000-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
<b>Year</b>									
First year	155.72 a	90.21 a	6.13 a	188.15 a	15.97 b	3.46 b	3429.12 a	13647.20 a	26.40 a
Second year	152.16 a	92.86 a	5.50 b	178.21 a	22.64 a	3.70 a	3238.72 b	13668.59 a	23.84 b
<b>Bacteria</b>									
PSf <sub>1</sub>	152.8 a	87.48 a	5.79 a	179.9 a	19.02 a	3.65 a	3362 b	13850 a	24.53 a
PSf <sub>2</sub>	155.7 a	90.22 a	5.86 a	176.4 a	19.77 a	3.65 a	3481 ab	13850 a	25.69 a
PSP <sub>1</sub>	154.9 a	109 a	5.85 a	177.8 a	18.78 a	3.48 b	3218 c	13500 ab	25.46 a
PSP <sub>2</sub>	152.6 a	84.66 a	5.78 a	182.1 a	19.10 a	3.65 ab	3587 a	14410 a	25.39 a
PS <sub>0</sub>	153.7 a	86.34 a	5.79 a	199.7 a	19.86 a	3.46 b	3055 d	12680 b	24.75 a
<b>Cultivar</b>									
Hayula 401	147.28 b	90.22 b	6.13 a	193.08 a	15.98 b	3.79 a	3426.50 a	13105 b	26.81 a
Sarigol	160.30 a	92.86 a	5.50 b	173.28 b	22.65 a	3.37 b	3254.25 b	14210 a	23.52 b
<b>Phosphorous</b>									
P <sub>1</sub>	157.4 a	102.50 a	5.77 a	176.5 b	19.73 a	3.72 a	3570 b	14480 a	25.46 ab
P <sub>2</sub>	155.3 a	90.04 a	5.82 a	207.2 a	19.50 a	3.67 a	3711 a	14720 a	25.83 a
P <sub>3</sub>	154.6 a	88.68 a	5.87 a	182.3 ab	19.02 a	3.52 b	3229 b	13330 b	25.00 a
P <sub>4</sub>	148.4 b	84.96 a	5.79 a	166.7 b	18.99 a	3.40 b	2852 d	12110 c	24.36 a

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

In the bacteria level, the greatest 1,000-grain weight was observed in PSf<sub>1</sub> and PSf<sub>2</sub>, and the lowest 1,000-grain weight was obtained in PSP<sub>1</sub> and PS<sub>0</sub>. The highest seed yield (3,587 kg ha<sup>-1</sup>) was produced in PSP<sub>2</sub> and the lowest seed yield (3,055 kg ha<sup>-1</sup>) was achieved in PS<sub>0</sub>. The maximum biological yields were equal to 13,850, 13,850, and 14,410 kg ha<sup>-1</sup>, which were produced for PSf<sub>1</sub>, PSf<sub>2</sub>, and PSP<sub>2</sub>, respectively (*Table 2*). The result of cultivar comparison showed that plant height and the height of the first pod in Sarigol were 8.84, 2.93, and 41.74 % more than Hayula 401, which causes an increase of 8.43 % in the biological yield. However, pod length, number of pods per plant, and the 1,000-seed weight for Hayula 401 were 11.45, 11.42, and 12.46 % more than Sarigol, thus bringing about the highest seed yield (3,426.5 kg ha<sup>-1</sup>) and the harvest index equal to 26.81 % (*Table 2*). The mean comparison of phosphorous treatment showed that the maximum plant height was observed in P<sub>1</sub>, P<sub>2</sub>, and P<sub>3</sub>, but the largest number of pods per plant (207.2 pods) was observed in P<sub>3</sub>, which caused the highest seed yield (3,711 kg ha<sup>-1</sup>). The greatest 1,000-seed weight and biological yield were obtained in P<sub>1</sub> and P<sub>2</sub>, respectively. The highest harvest index was achieved in P<sub>2</sub>, P<sub>3</sub>, and P<sub>4</sub> (*Table 2*).

Result of year × bacteria interaction showed that the highest plant heights (158.2 and 157.7 cm) were obtained in Y<sub>1</sub>PSf<sub>2</sub> and Y<sub>1</sub>PSP<sub>2</sub>, respectively. The greatest height of the first pod (123.3 cm) was observed in Y<sub>2</sub>PSP<sub>1</sub>. The maximum number of grains per pod (23.40 pods) was obtained in Y<sub>2</sub>PSf<sub>2</sub>. The greatest seed yield (3,670 kg ha<sup>-1</sup>) was produced in Y<sub>2</sub>PSP<sub>2</sub> and the highest harvest index was observed in Y<sub>1</sub>PSP<sub>1</sub> (*Table 3*). The year × cultivar result revealed that the greatest plant height (167.3 cm) and the

greatest height of pod (106.4 cm) were obtained in Y<sub>1</sub>C<sub>2</sub>. The highest pod length (6.2 cm), seed yield (3,517 kg ha<sup>-1</sup>), and harvest index (30.08 %) were produced in Y<sub>1</sub>C<sub>1</sub>. The greatest number of grains per pod (24.77 grains) and 1,000-seed weight (4.03 g) were achieved in Y<sub>2</sub>C<sub>1</sub> (Table 4).

**Table 3.** Interaction of year × bacteria on quantities and qualities yield of rapeseed cultivars

Interaction	Plant height (cm)	Height of first pod (cm)	Number of grain per pod	1000-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Harvest index (%)
Y <sub>1</sub> PSf <sub>1</sub>	154.8 ab	88.57 b	16.55 c	3.53 c	528 ab	26.02 b
Y <sub>1</sub> PSf <sub>2</sub>	158.2 a	96.65 ab	16.15 c	3.50 c	557 ab	25.62 bc
Y <sub>1</sub> PSp <sub>1</sub>	157.7 a	94.60 ab	15.17 c	3.38 c	3462 b	29.11 a
Y <sub>1</sub> PSp <sub>2</sub>	152.9 ab	83.50 b	15.34 c	3.45 c	500 ab	25.66 bc
Y <sub>1</sub> PS <sub>0</sub>	154.9 ab	87.75 b	16.66 c	3.44 c	141 cd	25.79 bc
Y <sub>2</sub> PSf <sub>1</sub>	150.8 b	86.38 b	21.52 b	3.75 a	319 c	23.04 cd
Y <sub>2</sub> PSf <sub>2</sub>	153.2 ab	83.80 b	23.40 a	3.81 a	405 b	25.75 bc
Y <sub>2</sub> PSp <sub>1</sub>	152.0 ab	123.30 a	22.40 ab	3.58 bc	297 d	21.80 d
Y <sub>2</sub> PSp <sub>2</sub>	152.3 ab	85.82 b	22.87 ab	3.84 a	367 a	25.13 bc
Y <sub>2</sub> PS <sub>0</sub>	152.5 ab	84.94 b	23.05 ab	3.48 c	296 d	23.71 b-d

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

**Table 4.** Interaction of year × cultivar on quantities and qualities yield of rapeseed cultivars

Interaction	Plant height (cm)	Height of first pod (cm)	Pod length (cm)	Number of pod per plant	Number of grain per pod	1000-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
Y <sub>1</sub> C <sub>1</sub>	144.1 c	74.04 b	6.20 a	182 a	17.23 c	3.55 b	3517 a	11900 d	30.08 a
Y <sub>1</sub> C <sub>2</sub>	167.3 a	106.40 a	6.06 ab	194.3 a	14.72 d	3.38 c	3358 b	15400 a	22.81 b
Y <sub>2</sub> C <sub>1</sub>	150.6 b	90.35 ab	5.94 b	204.2 a	24.77 a	4.03 a	3336 b	14320 b	23.53 b
Y <sub>2</sub> C <sub>2</sub>	153.8 b	95.36 a	5.05 c	152.2 b	20.52 b	3.36 c	3151 c	13020 c	24.24 b

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

The interaction of year × phosphorous result showed that the maximum height of pod (114.1 cm) was observed in Y<sub>2</sub>P<sub>1</sub>. The pod length and the harvest index in the first year for all the phosphorous treatment was higher than the second-year phosphorous treatment results (Table 5). The number of grains per pod in the second year for the phosphorous levels was more than the first year. The greatest seed yield (3,770 kg ha<sup>-1</sup>) was produced in Y<sub>1</sub>P<sub>2</sub> and the least seed yield (2,695 kg ha<sup>-1</sup>) was achieved in Y<sub>2</sub>P<sub>4</sub> (Table 5). The result of bacteria × cultivar interaction showed that the plant height of Sarigol in the bacteria level was more than Hayula 401 (Table 6). The greatest height of pod (112.6 cm) was observed in PSp<sub>1</sub>C<sub>1</sub>. The maximum pod length was achieved for Hayula 401 in PSf<sub>1</sub> and PSp<sub>1</sub>. The highest number of pods per plant (211.1 pods) was obtained for Sarigol in PS<sub>0</sub>. The maximum 1,000-seed weight (3.91 g) was obtained in PSp<sub>2</sub> for Hayula 401. The greatest seed yield (3,680 kg ha<sup>-1</sup>) was produced for Hayula 401 in PSp<sub>2</sub> and the least seed yield (2,943 kg ha<sup>-1</sup>) was obtained in PS<sub>0</sub> for Sarigol. The

most and the least harvest index (27.79 and 21.71 %) was obtained for PSp<sub>0</sub> in Hayula 401 and Sarigol cultivars, respectively (Table 6).

**Table 5.** Interaction of year × phosphorous on quantities and qualities yield of rapeseed cultivars

Interaction	Plant height (cm)	Height of first pod (cm)	Pod length (cm)	Number of pod per plant	Number of grain per pod	1000-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
Y <sub>1</sub> P <sub>1</sub>	157.8 a	90.82 ab	6.02 a	183.3 b	17.06 b	3.59 b	3644 ab	14420 ab	26.49 a
Y <sub>1</sub> P <sub>2</sub>	157.7 a	91.46 ab	6.19 a	228 a	16.17 bc	3.48 bc	3770 a	14740 a	26.70 a
Y <sub>1</sub> P <sub>3</sub>	154.7 a	88.54 ab	6.13 a	181.8 b	14.99 c	3.14 bc	3328 c	13180 cd	26.51 a
Y <sub>1</sub> P <sub>4</sub>	152.8 a	90.03 ab	6.08 a	159.6 b	15.67 c	3.36 c	3008 d	12250 de	26.07 a
Y <sub>2</sub> P <sub>1</sub>	157.1 a	114.10 a	5.42 b	169.8 b	22.39 a	3.86 a	3495 b	14530 ab	24.44 ab
Y <sub>2</sub> P <sub>2</sub>	152 a	88.63 ab	5.45 b	186.5 b	22.84 a	3.86 a	3652 ab	14700 a	24.95 ab
Y <sub>2</sub> P <sub>3</sub>	154.5 a	88.83 ab	5.61 b	182.9 b	23.05 a	3.16 b	3131 d	13470 bc	23.49 b
Y <sub>2</sub> P <sub>4</sub>	144.1 b	79.88 b	5.50 b	173.7 b	22.32 a	3.43 bc	2695 e	11970 e	22.66 b

\*Values within a column followed by the same letter are not significantly different at Duncan (P ≤ 0.05)

**Table 6.** Interaction of bacteria × cultivar on quantities and qualities yield of rapeseed cultivars

Interaction	Plant height (cm)	Height of first pod (cm)	Pod length (cm)	Number of pod per plant	Number of grain per pod	1000-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
PSf <sub>1</sub> C <sub>1</sub>	145.2 b	76.56 ab	6.10 a	196.08 b	20.58 a	3.77 ab	3468 bc	13760 ab	25.63 a-d
PSf <sub>1</sub> C <sub>2</sub>	160.4 a	98.40 ab	5.48 b	163.90 ab	17.49 b	3.52 cd	3256 d	13940 ab	23.43 de
PSf <sub>2</sub> C <sub>1</sub>	149.8 b	78.43 ab	6.09 a	197.50 ab	21.47 a	3.93 a	3521 ab	13010 bc	27.35 ab
PSf <sub>2</sub> C <sub>2</sub>	161.7 a	102 ab	5.62 b	155.3 b	18.08 b	3.37 de	3440 bc	14690 a	24.02 cde
PSp <sub>1</sub> C <sub>1</sub>	146.3 b	112.60 a	6.04 a	191.8 ab	21.01 a	3.68 bc	3299 cd	12940 bc	26.36 abc
PSp <sub>1</sub> C <sub>2</sub>	163.5 a	105.30 ab	5.67 b	163.8 ab	17.56 b	3.29 e	3138 d	14070 ab	24.55 bcd
PSp <sub>2</sub> C <sub>1</sub>	145.7 b	71.85 b	6.07 a	191.8 ab	21.34 a	3.91 a	3680 a	14100 ab	26.88 ab
PSp <sub>2</sub> C <sub>2</sub>	159.5 a	97.47 ab	5.49 b	172.3 ab	16.87 b	3.38 de	3494 b	14720 a	23.91 cde
PS <sub>0</sub> C <sub>1</sub>	149.7 b	71.48 b	6.05 a	188.3 ab	21.61 a	3.66 bc	3166 d	11720 c	27.79 a
PS <sub>0</sub> C <sub>2</sub>	157.7 a	101.20 ab	5.53 b	211.1 a	18.11 b	3.27 e	2943 e	13630 ab	21.71 e

\*Values within a column followed by the same letter are not significantly different at Duncan (P ≤ 0.05)

The result of the bacteria × phosphorous interaction revealed that the highest plant heights of 159.3 and 160.8 cm were observed in PSp<sub>1</sub> for P<sub>1</sub> and P<sub>3</sub>, respectively. The maximum number of pods per plant (269.4 pods) was achieved in PS<sub>0</sub>P<sub>1</sub>. The greatest 1,000-seed weight (3.89 g) was observed in PSf<sub>2</sub>P<sub>2</sub>. The greatest seed yield (4,202 kg ha<sup>-1</sup>) and biological yield (16,190 kg ha<sup>-1</sup>) were produced in PSp<sub>2</sub>P<sub>2</sub>. The least seed yield (2,648 kg ha<sup>-1</sup>) was observed in PS<sub>0</sub>P<sub>4</sub> (Table 7). The result of cultivar–phosphorous interaction demonstrated the maximum plant heights of 162.9 cm and 162.2 cm, which were observed for the Sarigol cultivar in P<sub>1</sub> and P<sub>2</sub>, respectively. The greatest pod length and number of grains per pod were obtained in all the phosphorous treatment level for

Hayula 401. The greatest seed yield (3,802 kg ha<sup>-1</sup>) was produced in P<sub>2</sub> for Sarigol cultivar, but the least seed yield (2,801 kg ha<sup>-1</sup>) was achieved for Sarigol in P<sub>4</sub> (Table 8).

**Table 7.** Interaction of bacteria × phosphorous on quantities and qualities yield of rapeseed cultivars

Interaction	Plant height (cm)	Number of pod per plant	Number of grain per pod	1000-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )
PSf <sub>1</sub> P <sub>1</sub>	155.6 abc	174.7 b	20.50 ab	3.86 ab	3495 d-g	14630 a-d
PSf <sub>1</sub> P <sub>2</sub>	155.7 abc	185.9 b	18.75 ab	3.74 abc	3807 bc	15170 abc
PSf <sub>1</sub> P <sub>3</sub>	153.7 a-d	182.9 b	18.85 ab	3.52 b-f	3239 ghi	13570 b-f
PSf <sub>1</sub> P <sub>4</sub>	146.3 d	176.3 b	18.03 a	3.44 c-f	2908 jk	12020 fg
PSf <sub>2</sub> P <sub>1</sub>	158.4 d	162.6 b	19.67 ab	3.64 a-e	3640 cde	14150 a-e
PSf <sub>2</sub> P <sub>2</sub>	156.3 abc	182.9 b	20.58 a	3.89 a	3995 ab	14900 abc
PSf <sub>2</sub> P <sub>3</sub>	154.5 a-d	192.6 b	19.33 ab	3.57 a-e	3368 f-i	13690 b-f
PSf <sub>2</sub> P <sub>4</sub>	153.6 a-d	167.4 b	19.50 ab	3.51 c-f	2920 jk	12670 d-g
PSp <sub>1</sub> P <sub>1</sub>	159.3 a	178 b	18.78 ab	3.69 a-d	3598 c-f	15210 ab
PSp <sub>1</sub> P <sub>2</sub>	153.4 a-d	194.7 b	18.67 ab	3.54 a-f	3333 f-i	14240 a-e
PSp <sub>1</sub> P <sub>3</sub>	160.8 a	179.4 b	18.78 ab	3.38 def	3120 ij	12750 d-g
PSp <sub>1</sub> P <sub>4</sub>	146 d	159.1 b	18.90 ab	3.32 ef	2823 kl	11810 fg
PSp <sub>2</sub> P <sub>1</sub>	159.9 ab	190.5 b	18.96 ab	3.67 a-d	3726 cd	14590 a-d
PSp <sub>2</sub> P <sub>2</sub>	153.8 a-d	203.2 b	19.73 ab	3.69 a-d	4202 a	16190 a
PSp <sub>2</sub> P <sub>3</sub>	152.7 a-d	172.5 b	18.73 ab	3.73 abc	3457 e-h	14240 a-e
PSp <sub>2</sub> P <sub>4</sub>	147.1 cd	162.1 b	18.73 ab	3.50 c-f	2962 jk	12620 d-g
PS <sub>0</sub> P <sub>1</sub>	157 ab	269.4 a	19.00 ab	3.75 abc	3389 e-i	13800 b-f
PS <sub>0</sub> P <sub>2</sub>	157.2 ab	184.2 b	20.73 a	3.49 c-f	3221 hi	13100 c-f
PS <sub>0</sub> P <sub>3</sub>	151.3 a-d	168.4 b	19.79 ab	3.40 c-f	2962 jk	12390 efg
PS <sub>0</sub> P <sub>4</sub>	149.2 bcd	59.29 c	19.53 ab	3.22 f	2648 l	11410 g

\*Values within a column followed by the same letter are not significantly different at Duncan (P ≤ 0.05)

**Table 8.** Interaction of cultivar × phosphorous on quantities and qualities yield of rapeseed cultivars

Interaction	Plant height (cm)	Pod length (cm)	Number of pod per plant	Number of grain per pod	1000-seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )	Harvest index (%)
C <sub>1</sub> P <sub>1</sub>	151.9 cd	5.99 a	187 ab	21.39 a	3.95 a	3678 ab	14090 ab	26.87 a
C <sub>1</sub> P <sub>2</sub>	147.4 d	6.08 a	206.3 a	21.16 a	3.88 a	3802 a	14210 ab	27.26 a
C <sub>1</sub> P <sub>3</sub>	149.7 d	6.14 a	193.7 a	20.79 a	3.76 a	3323 c	12740 d	26.91 a
C <sub>1</sub> P <sub>4</sub>	140.1 e	6.08 a	185.3 ab	20.66 a	3.58 b	2903 e	11380 e	26.18 ab
C <sub>2</sub> P <sub>1</sub>	162.9 a	5.55 b	166.1 ab	18.06 b	3.50 b	3461 c	14860 ab	24.06 bc
C <sub>2</sub> P <sub>2</sub>	162.2 a	5.56 b	208.1 a	17.85 b	3.47 b	3620 b	15230 a	24.39 bc
C <sub>2</sub> P <sub>3</sub>	159.5 ab	5.61 b	170.9 c	17.35 b	3.28 c	3135 d	13920 bc	23.09 c
C <sub>2</sub> P <sub>4</sub>	156.6 bc	5.51 b	148 c	17.32 b	3.22 c	2801 e	12830 cd	22.55 c

\*Values within a column followed by the same letter are not significantly different at Duncan (P ≤ 0.05)

Investigating the plant height, we observed that phosphorus fertilizer influenced this trait, and the lowest plant height belonged to phosphorous control treatment and all the levels of phosphorus fertilizer consumption stood in the same group without a significant difference. Therefore, it can be understood that with an increase in phosphate consumption to 50 % of the soil test, the plant height increased, and despite canola's greater height in phosphorus fertilizer consumption that was equal to the soil test, it did not show a significant difference in comparison to the treatment of phosphorus consumption up to 50 % of the soil test. It seems that the extra consumed phosphorus did not influence the canola's height. These results are consistent with the results of Malakuti and Sepehr (2002), who state that the excessive use of phosphorus will not eventually lead to the increase of crop growth, but it will gradually fixate in soil, and in the situation of water shortage and dryness, it will cause drought stress. The results of this investigation are inconsistent with the results of Asghar et al. (2004), who stated that the inoculation of the seeds of canola cultivars (*Brassica Juncea*) with different rhizosphere bacteria brought about a greater height of the plant (up to 5.56 %) in comparison to the non-inoculated seeds; this inconsistency can be a result of the differences in the climate and the soil of the experimental region or the different cultivated cultivars. In addition, it can be determined that canola cultivars are different in the height of the first pod from the ground surface and this factor is mostly controlled by the plant's genetic factor. Investigating the characteristic related to the height of the first capsule from the ground surface reveals that the type of the cultivars influenced the characteristic of the height of the first pod from the ground surface, and Sarigol, with 100.88 cm, had the biggest height and Hayula 401, with 82.19 cm, had the lowest height of the first capsule from the ground surface. Results showed that different cultivars differ in the height of the first capsule from ground, and the reason behind this difference can be that the cultivar Sarigol grows higher than the cultivar Hayula 401; therefore, its first capsule is also brings about a taller height.

In terms of pod length result, it can be understood that pod length is a characteristic mostly controlled by genetic factors and less affected by environmental factors. Karper and Andri (1991) showed that the number of blooming branches increases due to the application of phosphorus; moreover, phosphorus application in the flowering period, and increases the total dry weight and the number and the dry weight of the capsule. According to the mean comparison, the consumption of phosphorus fertilizer was 25 % lower than the soil test, with an average of 207.2 pods had the most pod obtained in phosphorous control treatment with 166.7 pods, which had the fewest pods per plant. This way, these demonstrated a significant difference with the treatment of no phosphorus fertilizer consumption, and with its genetic advantage, the cultivar Hayula 401 produced more capsules than the cultivar Sarigol. Bacteria levels could not have a significant effect because of the absorption of the needed phosphorus by the cultivars of canola.

In fact, the pod length of Hayula 401 was 20 % more than that of Sarigol, which can be due to the genetic differences present between these two types of canola; the mentioned characteristic was less influenced by non-genetic factors. Pod length in canola is mostly controlled by genetic structure and increase of capsule length, which leads to the increased number of seeds in capsule. This was consistent with our findings and the findings of Kim et al. (1996), who stated that the difference in pod length could be due to climatic situations as well. The number of seeds per pods seems to be extremely dependent on pod length; therefore, the longer the pods, the more seeds a pod

can contain. Hayula 401 exceeded Sarigol both in the characteristic of capsule length and the characteristic of seed number. However, other researchers stated that their findings were consistent with the results of Asghar et al. (2004), who had said the inoculation of the seeds of some canola cultivars (*Brassica Juncea*) with different rhizosphere bacteria increases the weight of a thousand seeds (up to 33.9 %) compared to the non-inoculated seeds. Investigating the effects of the application method of the phosphorus-solvent bacteria and its relationship with the consumption of the chemical fertilizer ammonium phosphate in canola's autumn cultivation, Madani et al. (2010) stated that they observed significant changes in the weight of a thousand seeds due to the use of different amounts of ammonium phosphate.

Phosphorus consumption equal with the soil test and 25 % less than the soil test are categorized into Group "a" and the treatments of phosphorus fertilizer consumption 50 % less than the soil test and the control group are categorized into Group "b". Putenam et al. (1992) stated that the 1,000-seed weight is a constituent of the seed yield, which has an important role in stating the power and the potential of production, and is influenced by genetic and environmental factors. 1,000-seed weight is an index of a plant's power for the proportionality of reservoir request in the periods of seed filling, which can be increased by eliminating different stresses such as irrigation limitation. The results obtained from this experiment show that capsule length and the 1,000-seed weight in the cultivar Hayula were greater than the corresponding features in the cultivar Sarigol.

Phosphorus consumption 25 % less than the soil test showed the greatest yields, and phosphorous control treatment revealed the least yield in the unit of area. The application of the bacteria *Pseudomonas* IV (*Putida* 168) and the advantage of 17 % compared to the control treatment and categorization in Group "a" had the most seed yield per hectare; it stood in the same group as the bacteria *Pseudomonas* II (*Fluorescence* 93); the least seed yield was observed in the control treatment. The most seed yield belonged to the bacteria *Pseudomonas* IV (*Putida* 168) in the second year and the least seed yield belonged to the treatment without *Pseudomonas* bacteria consumption. Overall, the effect of bacteria on seed yield was more apparent in the first year than in the second year. The most seed yield belonged to *Pseudomonas* IV (*Putida* 168) and phosphorus fertilizer 25 % less than the soil test, and the least yield was obtained in the treatment of no bacteria and phosphorus fertilizer consumption. Furthermore, the results show that the bacteria *Pseudomonas* IV4 and II (*Putida* 168 and 93) cause more increase in yield than *Pseudomonas* I and IV (*Fluorescence* 41 and 99). Seed yield in the treatment of phosphorus fertilizer consumption 25 % less than the soil test was 30 % more than the treatment of no phosphorus fertilizer consumption, thus indicating an increased yield under the influence of phosphorus. With an increased amount of phosphorus fertilizer against the soil test, not only did not the seed yield per plant increase but we also observed decreased seed yield in canola. The excessive increase of an element in soil seems to disturb the absorption of other elements and decrease the crop yield. As canola's need for fertilizer compared to phosphorus was met with the treatment of phosphorus fertilizer 25 % less than the soil test in the experiment, thereby increasing the fertilizer level decreased the efficiency of canola root in absorbing other elements, and therefore, the yield declined. Hamilton et al. (1993) stated that an increase in the amount of phosphorus in soil would decrease the yield; this was due to the high proportion of phosphorus to zinc and phosphorus to iron, and the accumulation of molybdenum and cadmium in the plant tissues. Moreover, they stated

that canola reacts to application of phosphorus fertilizer when the soil phosphorus is less than 100 kg ha<sup>-1</sup>, which is consistent with the results of the present study. Furthermore, in other studies, it has been reported that hormone-producing and phosphate-solvent microorganisms increase phosphorus absorption and crop yield in soils deficient in phosphorus. Ramezani (2009), in his experiments on rice, corn, and other grains reported increased yield owing to the application of phosphate-solvent bacteria. Biological yield became significant at the statistical level of one percent at the levels of phosphorus fertilizer, bacteria, and cultivar. Lack of phosphorus fertilizer consumption produced a smaller biological yield than the treatments of fertilizer consumption; therefore, the consumption of phosphorus fertilizer 25 % less than the soil test had the most biological yield and phosphorus control treatment had the least biological yield. As the application of phosphorus fertilizer influences the straw yield, it also influences the biological yield.

### Qualitative traits

Qualitative traits, which include oil content, oil yield, shoot phosphorus concentration and absorption, and grain phosphorus concentration and absorption, showed significant differences in 5 % in bacteria, phosphorus, and cultivar treatment (Table 9). Except oil yield and grain phosphorus absorption, all the traits were significant at all interaction treatments. Oil content was significant at all double-interaction of treatment (Table 9).

**Table 9.** Mean square of quantities and qualities yield of rapeseed cultivars *pseudomonas fluorescence* bacteria and amounts of phosphorus

S.O.V.	DF	Oil content	Oil yield	Shoot P concentration	Shoot P absorption	Grain P concentration	Grain P absorption
Year (Y)	1	7.99	477365.35	0.0001	10.45	0.001	63.43
Error	4	4.67	283545.11	0.001	24.87	0.0001	41.35
Bacteria (B)	4	4.71 *	348784.71 *	0.31 *	4175.44 *	0.095 *	277.04 *
Y×B	4	5.82 *	119407.73 *	0.001 *	55.01 *	0.001 *	14.66
Cultivar (C)	1	37.13 *	526817.91 *	0.006 *	282.17 *	0.007 *	82.20 *
Y×C	1	1.91 *	6780.56 *	0.0001 *	2944.41 *	0.0001 *	0.78
B×C	4	1.23 *	11255.20 *	0.0001 *	45.98 *	0.001 *	1.35
Phosphorous (P)	3	29.56 *	1685639.59 *	0.03 *	1210.40 *	0.076 *	534.94 *
Y×P	3	0.27	12503.14 *	0.0001 *	11.07 *	0.001 *	4.45
B×P	12	1.04 *	41591.37 *	0.001 *	58.79 *	0.004 *	20.88 *
C×P	3	4.77 *	24287.02 *	0.0001 *	14.31 *	0.0001 *	2.69
B×C×P	12	1.17 *	1978.27	0.0010 *	10.49 *	0.001 *	0.67
Error	187	1.52	15487.44	0.0001	35.85	0.001	3.93
CV. (%)	-	3.18	9.58	4.22	21.31	6.75	11.79

\*\* and \*, respectively, significant in 1 % and 5 % level

Based on the main mean comparison of qualitative traits, the results indicated that in first year, all the qualitative traits were greater than second year (Table 10). In the year × bacteria interaction, the greatest oil content (36.76 %) was obtained in first year for

PSf<sub>1</sub>. Oil yield in this interaction was varied because of the different seed yield variations. The greatest shoot phosphorous content and absorption was observed in both years for PSp<sub>2</sub>. Grain phosphorous content and absorption was highest in the first year for PSp<sub>2</sub> (Table 11).

**Table 10.** Mean comparison of quantities and qualities yield of rapeseed cultivars *pseudomonas fluorescence* bacteria and amounts of phosphorus

S.O.V.	Oil content (%)	Oil yield (kg ha <sup>-1</sup> )	Shoot P concentration (%)	Shoot P absorption (kg ha <sup>-1</sup> )	Grain P concentration (%)	Grain P absorption (kg ha <sup>-1</sup> )
<b>Year</b>						
First year	39.00 a	1343.33 a	0.50 a	17.32 a	0.27 a	26.40 a
Second year	38.64 a	1254.13 b	0.50 a	16.29 b	0.27 a	23.84 b
<b>Bacteria</b>						
PSf <sub>1</sub>	39.17 a	1320 b	0.2440 c	26.08 c	0.4969 b	16.79 bc
PSf <sub>2</sub>	38.91 a	1354 ab	0.3181 b	33.01 b	0.4988 b	17.46 b
PSp <sub>1</sub>	38.97 a	1257 c	0.1946 e	20.17 a	0.5063 b	16.35 c
PSp <sub>2</sub>	38.68 ab	1389 a	0.3835 a	41.67 a	0.5600 a	20.07 a
PS <sub>0</sub>	38.35 a	1173 d	0.2035 c	19.57 d	0.4344 c	13.37 d
<b>Cultivar</b>						
Hayula 401	39.21 a	1343.50 a	0.2736 a	27.90 a	0.5046 a	17.32 a
Sarigol	38.43 a	1254 b	0.2639 b	28.31 a	0.5022 a	16.30 a
<b>Phosphorous</b>						
P <sub>1</sub>	39.75 a	1420 a	0.2837 b	30.84 a	0.5070 b	18.11 b
P <sub>2</sub>	38.95 b	1447 a	0.2897 a	32.26 a	0.5370 a	19.92 a
P <sub>3</sub>	38.40 c	1238 b	0.2635 c	27.10 b	0.5018 b	16.28 c
P <sub>4</sub>	38.17 c	1089 c	0.2382 d	22.20 c	0.4512 c	12.92 d

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

**Table 11.** Interaction of year  $\times$  bacteria on quantities and qualities yield of rapeseed cultivars

Interaction	Oil content (%)	Oil yield (kg ha <sup>-1</sup> )	Shoot P concentration (%)	Shoot P absorption (kg ha <sup>-1</sup> )	Grain P concentration (%)	Grain P absorption (kg ha <sup>-1</sup> )
Y <sub>1</sub> PSf <sub>1</sub>	39.76 a	1406 a	0.2521 c	26.30 c	0.4958 c	17.60 b
Y <sub>1</sub> PSf <sub>2</sub>	38.62 bcd	1374 a	0.3175 b	34.37 b	0.4975 c	17.78 b
Y <sub>1</sub> PSp <sub>1</sub>	39.25 ab	1361 a	0.1988 f	19.37 d	0.5067 c	17.60 b
Y <sub>1</sub> PSp <sub>2</sub>	39.07 a-d	1371 a	0.3812 a	40.20 a	0.5717 a	19.14 a
Y <sub>1</sub> PS <sub>0</sub>	38.30 d	1204 bc	0.1996 f	19.22 d	0.4363 d	13.79 d
Y <sub>2</sub> PSf <sub>1</sub>	38.59 bcd	1233 b	0.2358 d	25.87 c	0.4979 c	15.99 c
Y <sub>2</sub> PSf <sub>2</sub>	39.19 abc	1334 a	0.3187 b	31.65 b	0.5000 c	17.13 b
Y <sub>2</sub> PSp <sub>1</sub>	38.70 bcd	1154 c	0.1904 g	20.98 d	0.5058 c	15.10 c
Y <sub>2</sub> PSp <sub>2</sub>	38.30 d	1407 a	0.3858 a	43.14 a	0.5483 b	20.30 a
Y <sub>2</sub> PS <sub>0</sub>	38.40 cd	1142 c	0.2075 e	19.91 d	0.4325 d	12.95 d

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

Based on year  $\times$  cultivar interaction, the most oil content and oil yield (39.31 % and 1,385 kg ha<sup>-1</sup>) and grain phosphorous content and absorption (17.85 % and 17.85 kg ha<sup>-1</sup>) was observed in the first year for Hayula 401 cultivar. The highest shoot phosphorous content and absorption were produced in both years for both cultivars (Table 12).

**Table 12.** Interaction of year  $\times$  cultivar on quantities and qualities yield of rapeseed cultivars

Interaction	Oil content (%)	Oil yield (kg ha <sup>-1</sup> )	Shoot P concentration (%)	Shoot P absorption (kg ha <sup>-1</sup> )	Grain P concentration (%)	Grain P absorption (kg ha <sup>-1</sup> )
Y <sub>1</sub> C <sub>1</sub>	39.31 a	1385 a	23.31 c	23.31 c	17.85 a	17.85 a
Y <sub>1</sub> C <sub>2</sub>	38.70 b	1302 b	32.48 a	32.48 a	16.79 b	16.79 b
Y <sub>2</sub> C <sub>1</sub>	39.12 ab	1306 b	30.73 a	30.73 a	16.94 b	16.94 b
Y <sub>2</sub> C <sub>2</sub>	38.15 c	1202 c	25.89 b	25.89 b	15.65 c	15.65 c

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

The result of year  $\times$  phosphorous interaction showed that the most oil content (39.99 %) was achieved in first year for P<sub>1</sub>. The least oil content 37.98 % was obtained in second year for P<sub>4</sub>. The highest oil yields, 1,459 and 1,479 kg ha<sup>-1</sup>, were produced in the first year in P<sub>1</sub> and P<sub>2</sub>. The maximum shoot phosphorous content and absorption were obtained in both years for P<sub>2</sub>. The greatest grain phosphorous content (0.54 %) was achieved in both years for P<sub>2</sub>. The highest grain phosphorous absorption (19.72 kg ha<sup>-1</sup>) was observed in the second year for P<sub>2</sub> (Table 13).

**Table 13.** Interaction of year  $\times$  phosphorous on quantities and qualities yield of rapeseed cultivars

Interaction	Oil content (%)	Oil yield (kg ha <sup>-1</sup> )	Shoot P concentration (%)	Shoot P absorption (kg ha <sup>-1</sup> )	Grain P concentration (%)	Grain P absorption (kg ha <sup>-1</sup> )
Y <sub>1</sub> P <sub>1</sub>	39.99 a	1459 a	0.2857 ab	30.65 ab	0.5073 b	18.52 b
Y <sub>1</sub> P <sub>2</sub>	39.17 bc	1479 a	0.2897 a	32.13 a	0.5360 a	12.01 f
Y <sub>1</sub> P <sub>3</sub>	38.46 de	1281 c	0.2647 c	26.33 c	0.5063 b	16.88 c
Y <sub>1</sub> P <sub>4</sub>	38.36 de	1154 d	0.2397 d	22.47 d	0.4567 c	13.76 e
Y <sub>2</sub> P <sub>1</sub>	39.51 ab	1382 b	0.2817 b	31.03 ab	0.5067 b	17.70 bc
Y <sub>2</sub> P <sub>2</sub>	38.73 cd	1415 ab	0.2897 a	32.39 a	0.5380 a	19.72 a
Y <sub>2</sub> P <sub>3</sub>	38.32 de	1196 d	0.2623 c	27.88 bc	0.4973 b	15.68 d
Y <sub>2</sub> P <sub>4</sub>	37.98 e	1024 e	0.2370 d	21.94 d	0.4457 c	12.08 f

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

Bacteria  $\times$  cultivar interaction results showed that the maximum oil content (39.63 %) was obtained for Hayula 401 in PSf<sub>1</sub> (Table 14). The most oil yield (1,446 kg ha<sup>-1</sup>) was achieved for Hayula 401 in PSp<sub>2</sub>. The highest shoot phosphorous concentration (0.39 %) was obtained for Hayula 401 in PSp<sub>2</sub>. The greatest grain phosphorous content (0.51 and 0.57 %) was obtained for Hayula 401 in PSp<sub>1</sub> and PSp<sub>2</sub> (Table 14).

**Table 14.** Interaction of bacteria × cultivar on quantities and qualities yield of rapeseed cultivars

Interaction	Oil content (%)	Oil yield (kg ha <sup>-1</sup> )	Shoot P concentration (%)	Shoot P absorption (kg ha <sup>-1</sup> )	Grain P concentration (%)	Grain P absorption (kg ha <sup>-1</sup> )
PSf <sub>1</sub> C <sub>1</sub>	39.63 a	1379 ab	0.2488 e	26.33 d	0.5025 c	17.55 c
PSf <sub>1</sub> C <sub>2</sub>	37.82 bc	1261 c	0.2392 f	25.84 c	0.4913 c	16.04 de
PSf <sub>2</sub> C <sub>1</sub>	39.26 ab	1380 ab	0.3229 c	30.78 b	0.5037 c	17.81 c
PSf <sub>2</sub> C <sub>2</sub>	38.55 bcd	1329 bc	0.3133 d	35.24 e	0.4938 c	17.10 cd
PSp <sub>1</sub> C <sub>1</sub>	39.12 ab	1293 cd	0.1975 h	19.16 e	0.5092 a	16.87 cde
PSp <sub>1</sub> C <sub>2</sub>	38.83 bc	1222 d	0.1917 i	21.19 a	0.5033 b	15.83 e
PSp <sub>2</sub> C <sub>1</sub>	39.27 ab	1446 a	0.3912 a	41.06 a	0.5708 a	20.81 a
PSp <sub>2</sub> C <sub>2</sub>	38.10 cd	1332 bc	0.3758 b	42.28 a	0.5492 b	19.33 b
PS <sub>0</sub> C <sub>1</sub>	38.78 bc	1230 d	0.2075 g	17.76 e	0.4367 d	13.92 f
PS <sub>0</sub> C <sub>2</sub>	37.72 d	1117 e	0.1996 h	21.37 e	0.4321 d	12.82 f

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

Based on bacteria × phosphorous interaction, the result revealed that the maximum oil content (40.38 %) was obtained in PSf<sub>1</sub>P<sub>1</sub> (Table 15).

**Table 15.** Interaction of bacteria × phosphorous on quantities and qualities yield of rapeseed cultivars

Interaction	Oil content (%)	Oil yield (kg ha <sup>-1</sup> )	Shoot P concentration (%)	Shoot P absorption (kg ha <sup>-1</sup> )	Grain P concentration (%)	Grain P absorption (kg ha <sup>-1</sup> )
PSf <sub>1</sub> P <sub>1</sub>	40.38 a	1414 cd	0.2533 i	28.13 efg	0.4983 ghi	17.41 ef
PSf <sub>1</sub> P <sub>2</sub>	39.63 abc	1508 bc	0.2733 h	31.05 def	0.5350 cde	20.33 bcd
PSf <sub>1</sub> P <sub>3</sub>	38.57 cd	1249 efg	0.2458 i	26.45 fgh	0.5050 fgh	16.37 fgh
PSf <sub>1</sub> P <sub>4</sub>	38.13 d	1108 hij	0.2033 k	8.52 ijk	0.4492 kl	13.06 ij
PSf <sub>2</sub> P <sub>1</sub>	39.69 abc	1445 c	0.3317 f	34.78 cd	0.4858 hij	17.65 ef
PSf <sub>2</sub> P <sub>2</sub>	38.93 cd	1560 ab	0.3493 e	37.99 bc	0.5417 cd	21.64 b
PSf <sub>2</sub> P <sub>3</sub>	38.82 cd	1297 ef	0.3117 g	32.15 de	0.5075 e-h	17.12 efg
PSf <sub>2</sub> P <sub>4</sub>	38.18 d	1114 hij	0.2800 h	27.12 e-h	0.4600 jk	13.42 ij
PSp <sub>1</sub> P <sub>1</sub>	40.08 ab	1445 c	0.2211 j	25.79 fgh	0.5217 d-g	18.79 de
PSp <sub>1</sub> P <sub>2</sub>	39.15 bcd	1306 def	0.2050 k	22.35 hi	0.5325 c-f	17.74 ef
PSp <sub>1</sub> P <sub>3</sub>	38.55 cd	1202 fgh	0.1883 l	17.86 ijk	0.4983 ghi	15.51 gh
PSp <sub>1</sub> P <sub>4</sub>	38.12 d	1076 ij	0.1633 m	14.70 k	0.4725 ijk	13.35 ij
PSp <sub>2</sub> P <sub>1</sub>	39.67 abc	1478 bc	0.3900 b	42.32 b	0.5567 bc	20.72 bc
PSp <sub>2</sub> P <sub>2</sub>	38.60 cd	1620 a	0.4142 a	49.55 a	0.6208 a	25.23 a
PSp <sub>2</sub> P <sub>3</sub>	38.05 d	1317 de	0.3717 c	40.19 b	0.5700 b	19.69 cd
PSp <sub>2</sub> P <sub>4</sub>	38.42 d	1141 ghi	0.3583 d	34.62 cd	0.4925 hl	14.63 hi
PS <sub>0</sub> P <sub>1</sub>	38.94 cd	1321 de	0.2217 j	22.99 g-u	0.4725 ijk	15.98 fgh
PS <sub>0</sub> P <sub>2</sub>	38.44 d	1240 efg	0.2067 k	20.34 ij	0.4550 kl	14.62 hi
PS <sub>0</sub> P <sub>3</sub>	38.03 d	1127 hi	0.2000 k	18.88 ijk	0.4283 l	12.70 j
PS <sub>0</sub> P <sub>4</sub>	38 d	1006 j	0.1858 l	16.07 jk	0.3817 m	10.15 k

\*Values within a column followed by the same letter are not significantly different at Duncan ( $P \leq 0.05$ )

The maximum oil yield ( $1,620 \text{ kg ha}^{-1}$ ), shoot phosphorous content (0.41 %), shoot phosphorous absorption ( $49.55 \text{ kg ha}^{-1}$ ), grain phosphorous content (0.62 %), and grain phosphorous absorption ( $25.23 \text{ kg ha}^{-1}$ ) were achieved in PSp<sub>2</sub>P<sub>2</sub> (Table 15). The result of cultivar  $\times$  phosphorous interaction showed that the maximum oil content (40.42 %) was obtained in P<sub>1</sub> for Hayula 401 (Table 16). The greatest oil yield (1,487 and  $1,502 \text{ kg ha}^{-1}$ ) was observed in P<sub>1</sub> and P<sub>2</sub> for Hayula 401. The highest shoot and grain phosphorous content (0.3 and 0.54 %), and grain phosphorous absorption ( $20.56 \text{ kg ha}^{-1}$ ) were produced for Hayula 401 in P<sub>2</sub> (Table 16).

**Table 16.** Interaction of cultivar  $\times$  phosphorous on quantities and qualities yield of rapeseed cultivars

Interaction	Oil content (%)	Oil yield ( $\text{kg ha}^{-1}$ )	Shoot P concentration (%)	Shoot P absorption ( $\text{kg ha}^{-1}$ )	Grain P concentration (%)	Grain P absorption ( $\text{kg ha}^{-1}$ )
C <sub>1</sub> P <sub>1</sub>	40.42 a	1487 a	0.2883 b	29.94 ab	0.5117 b	18.85 b
C <sub>1</sub> P <sub>2</sub>	39.49 b	1502 a	0.2970 a	31.57 a	0.5443 a	20.56 a
C <sub>1</sub> P <sub>3</sub>	38.75 cd	1284 c	0.2687 d	26.15 cd	0.5087 b	16.97 c
C <sub>1</sub> P <sub>4</sub>	38.19 d	1109 e	0.2403 f	20.41 e	0.4537 c	13.19 e
C <sub>2</sub> P <sub>1</sub>	39.08 bc	1354 b	0.2790 c	31.74 a	0.5023 b	17.37 c
C <sub>2</sub> P <sub>2</sub>	38.41 d	1391 b	0.2823 c	32.94 a	0.5297 a	19.28 b
C <sub>2</sub> P <sub>3</sub>	38.06 d	1193 d	0.2583 e	28.06 a	0.4950 b	15.59 d
C <sub>2</sub> P <sub>4</sub>	38.15 d	1070 e	0.2360 e	24 d	0.4487 c	12.65 e

\*Values within a column followed by same the letter are not significantly different at Duncan ( $P \leq 0.05$ )

The highest seed oil percentage was obtained in the treatment of phosphorus fertilizer equal to the soil test and the lowest was obtained in the treatment of the phosphorous control treatment. Supplying the plant with its needed nutritional sources increases its protein and oil percentage; practicing the phosphorus fertilizer also increased the oil content in this experiment. Furthermore, since phosphorus enhances shooting and flowering, it has a role in increasing the formation of more capsules, and eventually, the formation of more seeds. As a result, with an increase in seeds, the oil content also increases. Since better nutrition leads to better photosynthesis during canola's growth, and since supplying the needed phosphorus also fills the pod and seed, it affects the percentage and the yield of canola oil. With regard to the oil yield, phosphorus levels became significant. The consumptions of phosphorus fertilizer equal to the soil test and 25 % less than the soil test did not demonstrate a significant difference with each other in the oil yield and the least oil yield was obtained in the phosphorous control treatment. Different levels of phosphorus led to significant changes in the seed phosphorus concentrations; therefore, the most seed phosphorus was obtained in the treatment of phosphorus fertilizer 0.25 % less than the soil test and the least seed phosphorus was obtained in the treatment of no phosphorus fertilizer consumption. The bacteria *Pseudomonas* IV in the presence of phosphorus fertilizer 0.25 % was less than the soil test was the most and the least belonged to the treatment of no bacteria consumption and no bacteria. The phosphorus content of the seeds and shoots became significantly different at the levels of cultivar, bacteria, and phosphorus. The bacteria *Pseudomonas* IV led to the greatest phosphorus percentage in the seeds and the shoots, and the treatment of zero bacteria consumption had the least amount of

phosphorus content. Other researchers showed that the seed yields and the nitrogen uptake of canola were significantly increased by bacterization. Besides nitrogen, the uptake of other nutrients was also increased by these microorganisms. In general, pyrite amendment along with bacterization improved yields and nutrient uptake in canola and wheat over other treatments without pyrite addition (Joseph et al., 2014). Both bacterial and fungal strains exhibiting P-solubilizing activity were detected by the formation of a clear halo around their colonies. Production of a halo on a solid agar medium should not be considered the sole test for P-solubilization. When the colonies grow without a halo after several replacements of the medium, an additional test in the liquid media to assay P-dissolution should be performed, and a few isolates that are obtained after such a rigorous selection should be further tested for the abundant production of organic acids; the isolates complying with these criteria should be tested on a model plant as the ultimate test for potential P-solubilization (Bashan et al., 2013). Madani et al. (2011) revealed that the highest rate of seed yield (9.9 t/ha) was recorded in dual fertilizing applied both in sowing time and after the overwintering stage of the rosette. The interaction effects of phosphorus-solubilizing bacteria and the ammonium phosphate fertilizer application did not have a significant effect on plant height, biomass yield, the number of siliques per plant, seed oil percentage, and seed yield. Interaction effects of phosphorus-solubilizing bacteria and ammonium phosphate fertilizer application were significant for phosphorus content in plant tissues.

The effect of the mentioned bacteria in increasing the function of plants, such as rice and canola, has been reported (Ramezanpour, 2010; Yasari and Patwardhan, 2007). Yasari et al. (2008) reported that inoculation of *Azotobacter* and *Spirillum* had increased the amount of canola oilseed. The purpose of this research was to investigate the effects of using different species of *P. fluorescent* as the solvent bacteria of the soil's insoluble phosphate on the function and the phosphorus content of the leaves and the seeds of the canola cultivars Hayula and Sarigol.

## Conclusion

The application of phosphorus-solubilizing bacteria alone as a basic phosphorus fertilizer could not add to the phosphate compounds in plant parts. An increase in phosphorus content in plant parts by using chemical phosphorus fertilizers alongside phosphorus-solubilizing bacteria could cause major effect availability of soil phosphorus for rapeseed. According to the results which were obtained from this experiment, it can be stated that the bacterial inoculation of plants with the bacteria *Pseudomonas Putida* improved growth and it was better than the bacteria *Pseudomonas Putida*. Moreover, with the consumption of the bacteria, we can decrease the phosphate fertilizer consumption by 25 %; in this research, the cultivar Hayula 401 was better than the cultivar Sarigol with regard to yield and yield constituents.

## REFERENCES

- [1] Afzal, A., Ashraf, M., Asad, S. A., Farooq, M. (2005): Effect of phosphate solubilizing microorganisms on phosphorus uptake, yield and yield traits of wheat (*Triticum aestivum* L.) in rainfed area. – International Journal of Agricultural Biology 7: 207-209.
- [2] Asghar, H. N., Zahir, Z. A., Arshad, M., Khaliq, A. (2002): Relationship between in vitro production of auxins by rhizobacteria and their growth promoting activities in rapeseed. – Biology and Fertility of Soils 35: 231-237.
- [3] Bashan, Y., Kamnev, A. A., de Bashan, L. E. (2013): A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. – Biology and Fertility of Soils 49: 1-2.
- [4] Chen, Y. P., Rekha, P. D., Arunshen, A. B., Lai, W. A., Young, C. C. (2006): Phosphate solubilizing bacteria from subtropical soil and their Tri-calcium phosphate solubilizing abilities. – Applied Soil Ecology 34: 33-41.
- [5] Dey, K. B. (1988): Phosphate Solubilizing Organisms in Improving Fertility Status. – In: Sen, S. P., Palit, P. (eds.) Biofertilizers, Potentialities and Problems. Plant Physiology Forum, pp. 237-248. Naya Prokash, Calcutta.
- [6] Dobbelaere, S., Croonenborghs, A., Thys, A., Ptacek, D., Okon, Y., Vanderleyden, Y. (2002): Effect of inoculation with wild type *Azospirillum brasilense* and *A. irakense* strains on development and nitrogen uptake of spring wheat and grain maize. – Biology and Fertility of Soils 36: 284-297.
- [7] Egamberdiyeva, D., Juraeva, D., Poberejskaya, S., Myachina, O., Teryuhova, P., Seydaliyeva, L., Aliev, A. (2003): Improvement of wheat and cotton growth and nutrient uptake by phosphate solubilising bacteria. – 26<sup>th</sup> Southern Conservation Tillage Conference, Raleigh, NC.
- [8] Fallah, A. (2006): Abundance and distribution of phosphate solubilizing bacteria and fungi in some soil samples from north of Iran. – 18<sup>th</sup> World Congress of Soil Science, July 9-15, 2006, Philadelphia, Pennsylvania, USA.
- [9] Hamilton, M. A., Westermann, D. T., James, D. W. (1993): Factors affecting Zn uptake in cropping systems. – Soil Science Society American Journal 57: 1310-1315.
- [10] Hedley, M. J., Mortvedt, J. J., Bolan, N. S., Syers, J. K. (1995): P Fertility Management in Agroecosystems. – In: Tiessen, H. (ed.) P in the Global Environment, Transfers, Cycles and Management, pp. 59-92. Wiley, Chichester.
- [11] Jilani, G., Akram, A., Ali, R. M., Hafeez, F. Y., Shamsi, I. H., Chaudhry, A. N., Chaudhry, A. G. (2007): Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. – Annals of Microbiology 57: 177-183.
- [12] Joseph, A. R., Kavimandan, S. K., Kolluru Tilak, V. B. R., Nain, L. (2014): Response of canola and wheat to amendment of pyrite and sulphur-oxidizing bacteria in soil. – Archives of Agronomy and Soil Sciences 60: 367-375.
- [13] Kang, S. C., Hat, C. G., Lee, T. G, Maheshwari, D. K. (2002): Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102. – Current Science 82: 439- 442.
- [14] Khan, K. S., Joergensen, R. G. (2009): Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. – Bioresource Technology 100: 303-309.
- [15] Madani, H., Malboobi, M. A., Bakhshkelarestaghi, K., Stoklosa, A. (2011): Biological and chemical phosphorus fertilizers effect on yield and P accumulation in rapeseed (*Brassica napus* L.). – Notulae Botanicae Horti Agrobotanici 40(2): 210-214.
- [16] Malakoti, M. J., Sepehr, A. (2002): Optimum Nutrition for Oilseeds. – Khaniran Press, Tehran.
- [17] Pazouki, M., Ajam Norouzi, H., Ghanbari, A., Dadashi, M. R., Dastan, S. (2017). Energy and CO<sub>2</sub> emission assessment of wheat production scenarios in central areas of

- Mazandaran province, Iran. – Applied Ecology and Environmental Research 15(4): 143-161.
- [18] Pradhan, N., Sukla, L. B. (2005): Solubilization of inorganic phosphate by fungi isolated from agriculture soil. – African Journal of Biotechnology 5: 850-854.
- [19] Ramezanpour, M. (2009): Identification of phosphate solubilizing *Pseudomonas* sp. of rice rhizosphere based on 16S rDNA genotyping. – Middle-East Journal of Scientific Research 4(4): 348-353.
- [20] Ramezanpour, M., Popov, Y., Khavazi, K., AsadiRahmani, H. (2010): Genetic diversity and efficiency of indole acetic acid production by the isolates of fluorescent *Pseudomonads* from rhizosphere of Rice (*Oryza sativa* L.). – American-Eurasian Journal of Agricultural & Environmental Sciences 7(1): 103-109.
- [21] Ramezanpour, M., Popov, Y., Khavazi, K., AsadiRahmani, H. (2011): Molecular genosystematic and hysiological characteristics of fluorescent *Pseudomonads* isolated from the rice rhizosphere of Iranian paddy fields. – African Journal of Agricultural Research 6(1): 145-151.
- [22] Rashid, M., Khalil, S., Ayub, N., Alam, S., Latif, F. (2004): Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. – Pakistan Journal of Biological Sciences 7: 187-196.
- [23] Rodriguez, H., Fraga, F. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. – Biotechnology Advances 17: 319-339.
- [24] Rosas, S. B., Andres, G. A., Rovera, M., Correa, N. S. (2006): Phosphate-solubilizing *Pseudomonas putidacan* influence the Rhizobia legume symbiosis. – Soil Biology and Biochemistry 38: 3502-3505.
- [25] Salimpour, S., Khavazi, K., Nadian, H., Besharati, H., Miransari, M. (2012): Canola oil production and nutrient uptake as affected by phosphate solubilizing and sulfur oxidizing bacteria. – Journal of Plant Nutrition 35: 1997-2008.
- [26] Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., Gobi, T. A. (2013): Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. – Springerplus 2: 587.
- [27] Verma, L. N. (1993) Biofertiliser in Agriculture. – In: Thampan, P. K. (ed.) Organics in Soil Health and Crop Production, p. 152-183. Peekay Tree Crops Development Foundation, Cochin.
- [28] Yasari, E., Patwardhan, A. M. (2007): Effects of Azotobacter and Azospirillum inoculations and chemical fertilizers on growth and productivity of Canola. – Asian Journal of Plant Science 6: 77-82.
- [29] Yasari, E., Esmaeli, A., Pirdashti, H., Mozafari, S. (2008): Azotobacter and Azospirillum inoculants as bio-fertilizers in canola (*Brassica napus* L.) cultivation. – Asian Journal of Plant Science 7(5): 490-494.
- [30] Yazdani, M., Bahmanyar, M. A., Pirdashti, H., Esmaili, M. A. (2009): Effect of Phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of Corn (*Zea mays* L.). – Proceedings of World Academy of Science, Engineering and Technology 37: 90-92.